The Fate of Pyrogenic Organic Matter in Soil and Its Interaction with Native Soil Organic Matter and Increased N Inputs

Dissertation zur Erlangung der

naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Bernardo Maestrini

aus

Italien

Promotionskomitee

Prof. Dr. Michael W. I. Schmidt (Vorsitz)

Dr. Samuel Abiven (Leitung der Dissertation)

Prof. Dr. Paolo Nannipieri

Zürich, 2014

[...]*Così una nuova molecola d'anidride carbonica ritornò all'atmosfera, ed una parcella dell'energia che il sole aveva ceduta al tralcio passò dallo stato di energia chimica a quello di energia meccanica e quindi si adagiò nella ignava condizione di calore, riscaldando impercettibilmente l'aria smossa dalla corsa ed il sangue del corridore. «Così è la vita», benché raramente essa venga così descritta: un inserirsi, un derivare a suo vantaggio, un parassitare il cammino in giù dell'energia, dalla sua nobile forma solare a quella degradata di calore a bassa temperatura. Su questo cammino all'ingiù, che conduce all'equilibrio e cioè alla morte, la vita disegna un'ansa e ci si annida*[...]

[...]So a new molecule of carbon dioxide returned to the atmosphere, and a parcel of the energy that the sun had handed to the vine-shoot passed from the state of chemical energy to that of mechanical energy, and thereafter settled down in the slothful condition of heat, warming up imperceptibly the air moved by the running and the blood of the runner. "'Such is life"', although rarely is it described in this manner: an inserting itself, a drawing off to its advantage, a parasitizing of the downward course of energy, from its noble solar form to the degraded one of low temperature heat. In this downward course, which leads to equilibrium and thus death, life draws a bend and nests in it[...]

P. Levi, R. Rosenthal (translator), Il Sistema Periodico, Carbonio

Summary

Pyrogenic organic matter (PyOM), the product of incomplete combustion of biomass, represents an important fraction of soil organic matter in natural and agricultural soils. In fact PyOM is produced either naturally by wildfires (charcoal) or artificially by pyrolysis of agricultural residues (biochar). It has been suggested, based on incubation experiments on PyOM mineralization and observations of PyOM radiocarbon age that PyOM has a long time residence time. However a large uncertainty still exists on PyOM loss processes and rate and on the possible interactions with the surrounding native soil organic matter. Therefore determining PyOM turnover time and the impact on native soil organic matter mineralization are key issues to understand the impact of PyOM input on the carbon (C) cycle.

The present work investigates the impact of PyOM on soil C budget, with a special regard to the effect of mineral nitrogen (N) deposition on PyOM mineralization. The present thesis is composed of three independent studies: (i) a field study to investigate the relative importance of PyOM loss processes , (ii) an incubation experiment to elucidate C and N dynamics in PyOM amended soil and, (iii) a meta-analysis of the priming effect induced by PyOM on soil organic C. Both in the field and in the incubation experiment the stable isotopes of C and N were used to measure organic matter mineralization.

In the field experiment it was observed that: (i) pyrolysis decreased by a factor of 60 pinewood decomposition (ii) N input decreased the mineralization of PyOM, (iii)Leaching and translocation of fresh PyOM along the soil profile were negligible compared to losses as CO_2.

In the incubation experiment the influence of ryegrass-derived PyOM on soil organic matter mineralization (priming effect) and the effect of mineral N input on PyOM mineralization were investigated. No effect of N input on PyOM mineralization was observed. The different response compared to the pinewood-derived PyOM is probably due its high N content in ryegrass-derived PyOM. Ryegrass-derived PyOM had a higher decomposition rate than pinewood-derived PyOM, confirming the applicability of the H:C ratio (an indicator for PyOM aromaticity) as a predictor of PyOM stability.

It was observed that PyOM induced a two-phase priming effect on native soil organic matter decomposition: on the short term PyOM induced positive priming while it induced negative priming in the long term. PyOM induced priming effect was further investigated in a meta-analysis that confirmed this temporal pattern. The meta-analysis showed that positive priming effect was induced mostly on the short term and by PyOM characterised by a low C content. It is possible that the presence of a labile fraction in PyOM triggers the activity of soil microorganisms and increase soil organic matter mineralization. In conclusion the present thesis shows that PyOM chemical composition and feedstock play an important role in predicting PyOM stability, and that the initial positive priming effect may decrease the efficiency of PyOM as a C-sink. Furthermore in the future the increase in N deposition may affect the stability of PyOM, probably depending on the availability of PyOM-N.

Zusammenfassung

Pyrogene organische Substanz (PyOM) ist das Produkt unvollständiger Verbrennung von Biomasse und trägt einen bedeutenden Anteil zur organischen Bodensubstanz in Wald- und Landwirtschaftsböden bei. PyOM wird entweder durch natürliche Brände (Holzkohle) oder durch künstliche Pyrolyse landwirtschaftlicher Nebenprodukte (Biokohle) erzeugt. Ergebnisse von Abbaustudien (Inkubationsexperimente) sowie alte Radiokarbonalter von PyOM weisen darauf hin, dass PyOM eine relativ lange Umsatzzeit besitzt. Es bestehen jedoch grosse Unsicherheiten bezüglich der Bedeutung der verschiedenen Verlustprozesse von PyOM aus Böden (Mineralisierung, Auswaschung), dessen Raten, sowie bezüglich möglichen Wechselwirkungen mit der restlichen (nativen) organischen Bodensubstanz. Um den Einfluss von PyOM auf den globalen Kohlenstoffkreislauf zu verstehen, ist es von entscheidender Bedeutung, die Umsatzzeiten von PyOM zu bestimmen, sowie dessen Einfluss auf die Mineralisierung nativer organischer Bodensubstanz zu erfassen. Die vorliegende Arbeit untersucht den Einfluss von PyOM auf den Bodenkohlenstoffhaushalt, unter spezieller Betrachtung der Auswirkungen von mineralischen Stickstoffeinträgen auf die Mineralisierung von PyOM. Die vorliegende Doktorarbeit besteht aus drei unabhängigen Studien: (i) eine Feldstudie um die relative Bedeutung der PyOM Verlustprozesse zu bestimmen, (ii) ein Inkubationsexperiment um die Kohlenstoff- und Stickstoffdynamik in Böden nach der Zugabe von PyOM zu untersuchen und (iii) eine Metaanalyse des Primingeffekts, einer Wechselwirkung zwischen PyOM und der nativen organischen Bodensubstanz.. Im Feld- sowie im Inkubationsexperiment wurde mit stabilen Isotopen markierte organische Substanz verwendet um dessen Mineralisierung zu untersuchen. Im Feldexperiment wurde beobachtet, dass (1) der Abbau von Pinienholz durch Pyrolyse um einen Faktor von 60 abnahm, dass (2) erhöhter Stickstoffeintrag die Mineralisierungsrate von PyOM herabsetzt und dass (3) Verluste durch Verlagerung von frischem PyOM entlang des Bodenprofils (Auswaschung) vernachlässigbar sind im Vergleich zu Verlusten durch Mineralisierung zu CO2. Im Inkubationsexperiment wurde der Einfluss von Weidelgras-PyOM auf die Mineralisierung der organischen Bodensubstanz (Primingeffekt) in Abhängigkeit der Stickstoffeinträge erfasst. Der Abbau von Weidelgras-PyOM wurde nicht von Stickstoffzugaben beeinflusst. Für Pinienholz-PyOM wurden allerdings unterschiedliche Effekte beobachtet, was möglicherweise auf den höheren Stickstoffgehalt in Weidelgrass-PyOM zurückzuführen ist. Weidelgrass-PyOM wies höhere Wasserstoff zu Kohlenstoff Verhältnisse (H/C) auf und hatte eine höhere Abbaurate als Pinienholz-PyOM, was die Anwendbarkeit des H/C-Verhältnisses (ein Indikator für PyOM Aromatizität) zur Vorhersage der PyOM Stabilität bestätigt. Des Weiteren ein zwei-Phasen-Primingeffekt von PyOM auf den Abbau der nativen organischen Bodensubstanz beobachtet: in kurzen Zeiträumen verursacht PyOM einen positiven Primingeffekt, während sie über längere Zeiträume einen negativen Primingeffekt verursacht. Das zeitliche Muster des von PyOM verursachten Primingeffekts konnte in der Metaanalyse bestätigt werden. Die Metaanalyse zeigte, dass positives Priming meistens in kurzen Zeiträumen stattfindet und von PyOM mit niedrigen Kohlenstoffgehalten verursacht wird. Eine mögliche Erklärung ist, dass die Präsenz einer labilen Fraktion in PyOM die Aktivität von Mikroorganismen beeinflusst und dadurch die Mineralisierung von organi-

schem Bodenkohlenstoff erhöht. Zusammenfassend konnte mit dieser Doktorarbeit gezeigt werden, dass die chemische Zusammensetzung und das Ausgangsmaterial von PyOM eine wichtige Rolle zur Vorhersage der PyOM-Stabilität spielen und dass anfänglich positives Priming möglicherweise die Effizienz von PyOM als Kohlenstoffsenke mindert. In Zukunft könnte zudem die Zunahme der Stickstoffeinträge die Stabilität von PyOM beeinflussen, wahrscheinlich in Abhängigkeit der Verfügbarkeit von PyOM-Stickstoff.

Contents

Chapter A

Synopsis

1 Introduction

1.1 The C cycle

The carbon (C) cycle consists of fluxes of C among C reservoirs. These are, in order of magnitude: oceans (approximately 40.000 Pg C), soils (approximately 2000 Pg C), permafrost (1700 Pg C) fossil fuel reserves (approximately 1000 Pg C), atmosphere (600 Pg C), vegetation (300 Pg C, IPCC, 2013). The quantity of C present in the atmosphere in the form of carbon dioxide (CO_2) is a determinant for the quantity of energy reaching the planetary surface. In fact CO_2, together with other gases is responsible for the absorption of infrared thermal radiation and its subsequent re-radiation to the earth surface, a phenomenon known as greenhouse effect. The concentration of CO_2 in the atmosphere, has risen by 40% since the pre-industrial era and is predicted to continue increasing over the next decades, inducing climate changes at global scale(IPCC, 2013).
Although the C stored in the soils is twenty times less than C stored in the oceans, C fluxes between the terrestrial ecosystems and the atmosphere and between the ocean and the atmosphere are in the same order of magnitude. Soils release globally 118 Pg C-CO_2 year^{-1} and are one of the main sources of CO_2 to the atmosphere (IPCC, 2013, Figure 1), therefore understanding the factors determining C exchanges between soils and the atmosphere are of primary importance to predict future changes and eventually reduce the emissions of CO_2 to the atmosphere.
A brief description of the C cycle in the terrestrial ecosystems is provided below: CO_2 is present in the atmosphere at a concentration of approximately 400 ppm, plants uptake CO_2 by photosynthesis and incorporate it as organic C. Plant C is transferred to the soil system by litterfall and rhizodeposition, part of the litter is directly respired as CO_2 by litter microorganisms, and part is incorporated in the soil and constitutes the soil organic matter pool (Figure 2). A large part of it is then mineralized as CO_2 by soil microorganisms, while a smaller fraction is leached to the belowground in form of dissolved organic C (DOC) or it is eroded. Typically the losses of organic C by mineralization are in the order of magnitude of hundreds of grams m^{-2} year^{-1} (Bond-Lamberty and Thomson, 2010), while

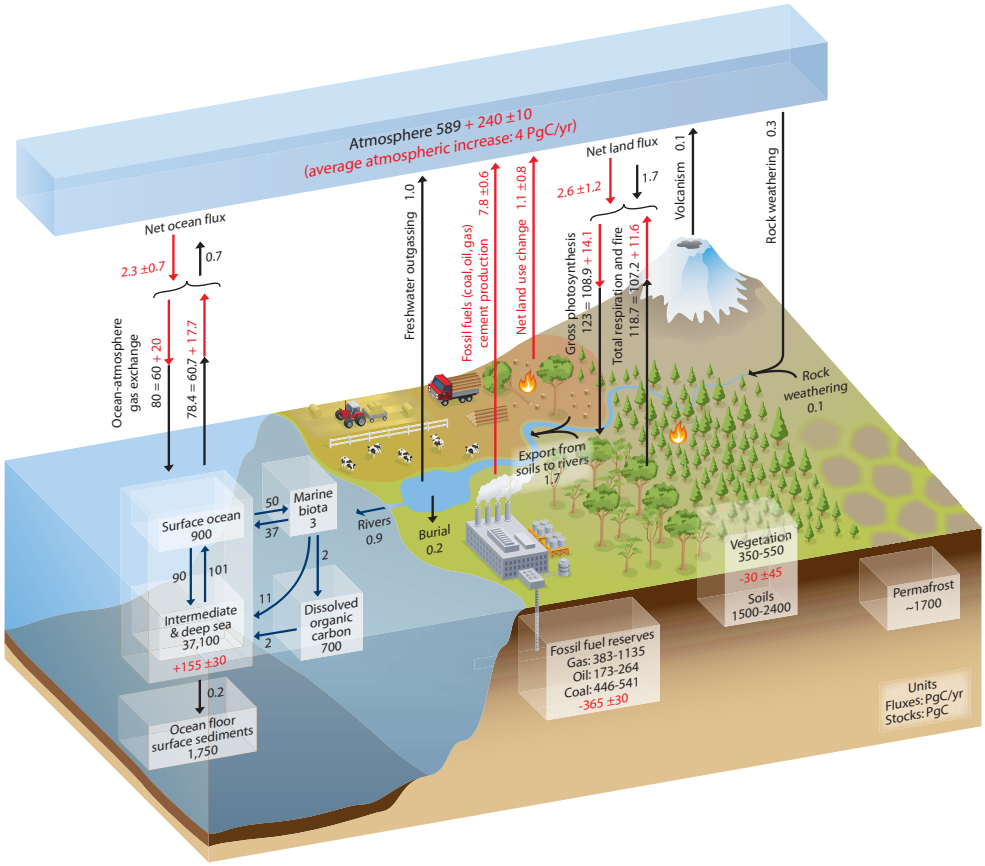


Figure 1: *The terrestrial C cycle. Arrows represents C fluxes, while boxes stand for C stocks. The black numbers and arrows indicate the estimated fluxes and stocks before the industrial era. The red arrows and numbers indicate the human-induced fluxes over the 2000-2009 (Intergovernmental Panel on Climate Change, 2013).*

losses as DOC are generally hundred times lower (Brady and Weil, 2007). Nevertheless these values differ significantly across different biomes and land uses. The CO_2 efflux from the soil takes the name of soil respiration, which can be divided into two CO_2 fluxes: the heterotrophic soil respiration, which is derived from microorganisms decomposing the soil organic matter and the autotrophic respiration, derived from the respiration of plant roots. The present study focuses on the heterotrophic soil respiration, as the interactions between plants and soil were not investigated in the present study. From here on, for sake of brevity, the term soil respiration refers to the heterotrophic fraction of soil respiration. Soil respiration, i.e. the organic matter mineralization, is driven by the interaction of environmental factors (soil temperature, humidity and structure) and chemical qualities of decomposing organic matter, e.g. the prevalence of aromatic or aliphatic compounds or the C:Nitrogen (N) ratio. Which of the two groups of factors, soil organic matter chemistry or environmental factors, is the main driver for stabilization of soil organic matter on the long term is still a matter of debate (Schmidt et al., 2011).

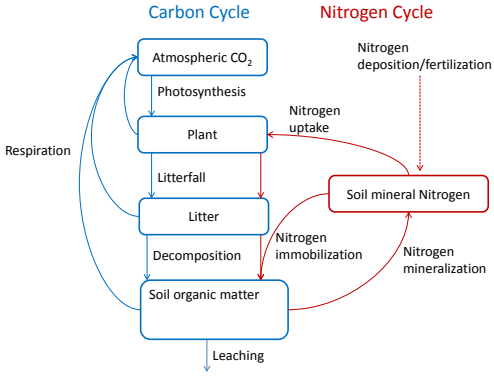


Figure 2: *A scheme representing C and N cycle, blocks represent stock of C and N and arrows represent the fluxes. The figures shows particularly how N can affect C cycle by altering plant growth (fertilization effect), and by influencing C decomposition in the soil (Thornton et al., 2009).*

1.2 The N cycle

Among the main elements constituting organisms (N, C, O, H and S) N is the most abundant on earth. However its availability to organisms is extremely limited, as most of it exist in the form of molecular N (N_2) and among the non-N_2 forms of nitrogen only 1% is plant available (Galloway et al., 2004).Therefore N is often the limiting factor for plants growth which are competing with microorganisms to uptake mineral N. The reactive N, a term indicating all forms of non-N_2 is present in two forms in the soil (Figure 3): the organic N, which includes living and dead organic matter and the mineral N (in form of $NH_4{}^+$, $NO_3{}^-$, NO_2^-). N inputs to the soil derive from plant residues, atmospheric mineral N deposition and fertilisation in agricultural soils. Organic matter represents the biggest N-pool (Chapin et al., 2002) and is mineralized by the joint action of microorganisms and extracellular enzymes to ammonium (NH_4^+). Extracellular enzymes are proteins released by soil microorganisms to cleave organic molecules, microorganisms release enzymes to depolymerize high molecular weight organic N (like proteins) into low molecular weight organic N forms (like oligo peptides) which can then be transformed in ($NH_4{}^+$) either by extracellular enzymes or microorganisms (mineralization process). NH_4^+ can be transformed by nitrifier microorganisms to nitrate (NO_3^-). The fate of mineral N ($NH_4{}^+$ and $NO_3{}^-$) in the soil is to be incorporated by microorganisms (immobilization process) or uptake by plants. Moreover N can also leave the soil system in gaseous form *(denitrification)*, a process mediated by microorganisms transforming $NO_3{}^-$ first into NO_2^- and then into N_2.
Immobilization and mineralization processes occur simultaneously in the soil. If overall gross mineralization processes are prevalent on gross immobilization processes this results in an increase of $NH_4{}^+$, i.e. in *positive net* mineralization, while if $NH_4{}^+$ in soil decrease the *net mineralization* is negative. When referring to immobilization and mineralization processes considered individually, and not as the sum of the two, we use the adjective *gross*.

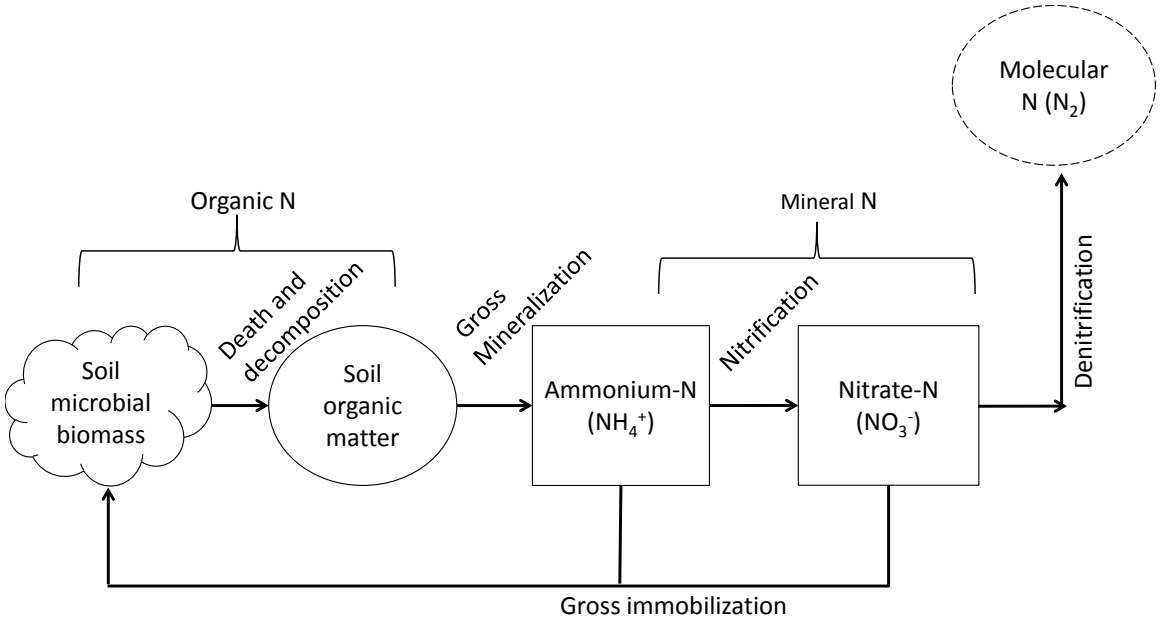


Figure 3: *N transformations in soil. Blocks represent stocks of N while arrows represent fluxes of N. The size of the blocks is not representative of the size of the N pools.*

1.3 Feedback of N cycle on C cycle

It is well known that N availability in the terrestrial system may dramatically influence the C cycle, however very few C models incorporate the feedback of N cycle on the C cycle (IPCC 2013) since the underneath mechanisms are still not fully understood. A full comprehension of these mechanisms is crucial to predict future changes in C cycle, especially because inputs of reactive N to the terrestrial system have sharply increased over the last century. The increase in N input follows from an increase of N fertilization, fossil fuel combustion, and cultivation of N fixing species (Figure 4). The Haber-Bosh process, an industrial process that transform N_2 to NH_3, together with cultivation of N fixing plants caused a human-induced increase in the N transfer from the non-reactive (N_2) to the reactive pool. Once reactive N has been added to the soil this is rapidly transferred to other environmental compartments like water bodies and atmosphere, and only partially returns to the molecular form through denitrification, with the result that reactive N is accumulating in the different environmental compartments. Part of the reactive N is transferred from the soil to the atmosphere in forms of NH_3, NO, N_2O transported by the wind and again deposited to terrestrial or aquatic system, i.e. it is redistributed in the form of N atmospheric deposition. The modelled global distribution of N deposition from the atmosphere (Figure 5) shows that the increase in deposition is concentrated in the more densely populated areas as energy and food production represents the two major source of N deposition.
The increase in N deposition may affect C cycle mostly by: (i) increasing photosynthetic

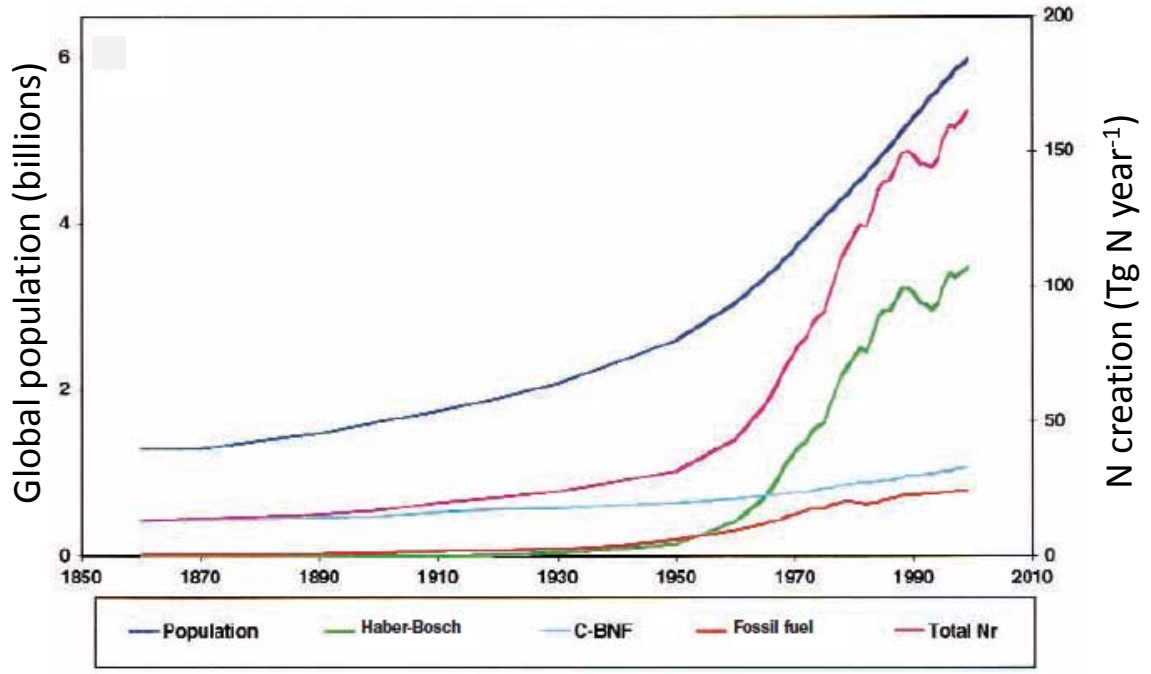


Figure 4: *Increase in reactive N and human population growth over the past century. The graph report the total N input and the three major input of N: the Haber-Bosh process (mineral N fertilization), the increase in cultivation of plants capable of fixing N (C-BNF), and the fossil fuel combustion (Galloway et al., 2003).*

C assimilation and (ii) by influencing biogeochemical processes and particularly the soil respiration (Figure 2). While there is a general agreement that increasing input of N will increase the photosynthetic C uptake, a major disagreement exists on the size of the fertilization effect (Magnani et al., 2007; De Schrijver et al., 2008), particularly under the scenario of future increased CO_2 atmospheric concentration (Reich and Hobbie, 2012; Schneider et al., 2004).
A high uncertainty exists on the impact on N availability on soil respiration (see section 1.7). The present study is focused on the impact of increased N input on soil organic matter mineralization, particularly on the mineralization of charcoal in the soil, while the impact of N inputs on plant growth was not investigated.

1.4 Impact of fire on the C cycle

Fire is a disrupting event that has a strong impact on the C cycle, particularly on the concentration of CO2 in the atmosphere. However producing a global assessment of the influence of wildfires on the C cycle is a hard challenge due to the heterogeneity of wildfire characteristics and return time. There is a general agreement among climate models in predicting an increase of fire activity in the future (Figure 6). Moritz et al. (2012) and Flannigan et al. (2013) compiled results from several climatic models and predicted an increase of fire-prone climatic conditions over the next decades. Particularly an increase of fire events is predicted in the high-to-mid-latitude-regions, as a result of increasing temperature in the

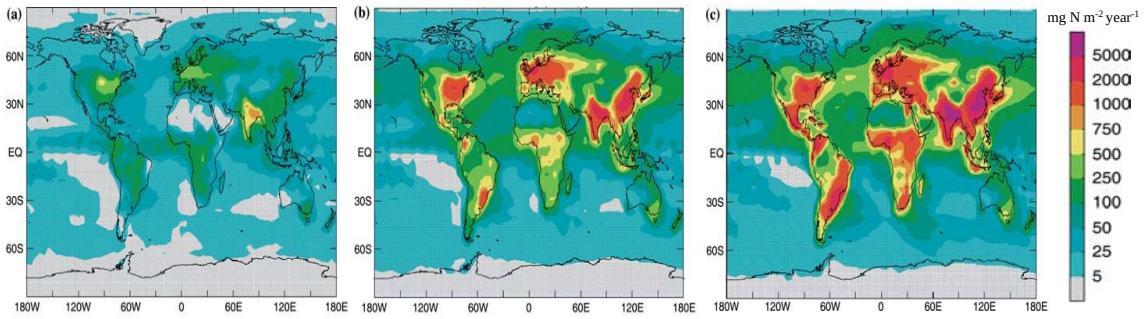


Figure 5: *Spatial patterns of modeled total inorganic nitrogen deposition in (a) 1860, (b) early 1990s, and (c) 2050 (Galloway et al., 2008)*

warm months, while a decrease is predicted in tropical regions following from a decrease in precipitation that will reduce the fuel loads.

Fire can greatly influence the C cycle mostly by releasing enormous quantity of C stored in plants and soil, ranging globally from 2 to 4 Pg C-CO_2 year^{-1} corresponding to approximately 50% of fossil fuel emissions (Bowman et al., 2009). Even though great advances in capacity of remotely measure the size of burnt area (Chuvieco, 1999) and fire severity (Miller et al., 2009) have been reached, there are still many uncertainties on the net effect of fire on C cycle. In fact next to the release of CO_2, there are at least two more feedback mechanisms that can influence C cycle after a fire event: (i) the plant regrowth after a fire event and (ii) the charcoal production. These two mechanisms are acting at a different time scale: CO_2 release from biomass burning is instantaneous, increase of plant growth rate occurs on a decadal time scale, while the storage of C in the form of charcoal in the soil is supposed to last for hundreds of years. Therefore the impact of fire on C budget is strongly dependent on the size of the considered time frame.

The present thesis is focused on the role played by charcoal (from here on Pyrogenic Organic Matter, PyOM) on the soil C budget, while the impact of fire on vegetation C losses was not investigated here, neither was the role of plant regrowth.

The first estimates of PyOM production at global scale dates back to the 1980s. Seiler and the nobel prize Crutzen (1980) estimated, based on values of fire frequency, burning efficiency, and total biomass, differentiated by biomes, that the annual global production of PyOM-C was 500-1700 Tg. Few years later Crutzen and Andreae (1990) revised it to be 200-600 Tg-PyOM-C year^{-1}. Kuhlbusch and Crutzen (1995), using a stricter definition of PyOM based on the H:C ratio (an indicator for aromaticity), reviewed it down to 40-180 Tg of PyOM-C year^{-1}. The C budget after a fire event is strongly affected by the burning efficiency, which is the ratio between the CO_2 and PyOM produced during a wildfire. Forbes et al. (2006) reviewed these values for different ecosystems but a clear picture of the factors controlling fire efficiency is still missing due to the heterogeneity of the methods to measure the PyOM remaining after a forest fires, and the very high spatial variability.

Due to its long residence time PyOM is virtually present in every soil and can contribute up

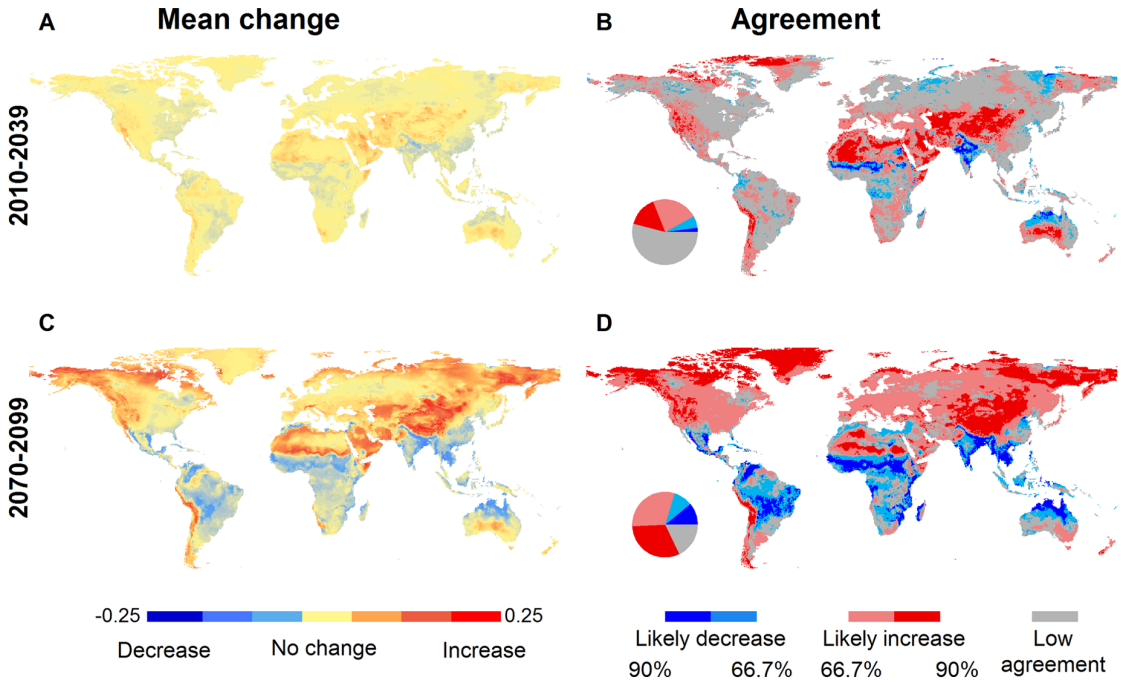


Figure 6: *Mean change (A, C) and degree of model agreement (B, D) in predicted fire probability among 16 Global Climate Models for 2010-2039 and 2070-2099 time periods (Moritz et al., 2012)*

to 45% of organic C present in soils (Schmidt and Noack, 2000). PyOM has not been detected only in soil, but also in sediments (Schmidt and Noack, 2000) and and water bodies, like oceans and rivers (Dittmar et al., 2012; Ziolkowski and Druffel, 2010), however very little is known on the processes that transport PyOM from the soil the other compartments, like water bodies (see section 1.8).

1.5 Characteristics of pyrogenic organic matter

PyOM is the product of incomplete combustion of biomass (Goldberg, 1985). Under non-limiting oxygen conditions, the burning process would transform all the organic material into CO_2, H_2O, and ashes, while temporary and local limitation of oxygen can inhibit the condensation process and produce organic residues, the PyOM. The first striking characteristic of PyOM is the color, it is generally pitch black (therefore it is also termed ”black carbon”). The macrostructure of PyOM is characterized by the presence of large porosity, derived from the loss of the internal part of the cell and the remaining of the cell walls (Figure 7). Such a high porosity is reflected also in a large surface area and high cations exchange capacity (Lehmann, 2007).
PyOM is a chemically heterogeneous compound, rich in aromatic molecules having different degrees of condensation (Schneider, 2011). The aromatic structure of PyOM has shown to be relatively resistant to chemical oxidants (Skjemstad et al., 1996; Bird and Grocke, 1997; Ascough et al., 2011), and highly resistant to biotic oxidation (Hamer et al., 2004;

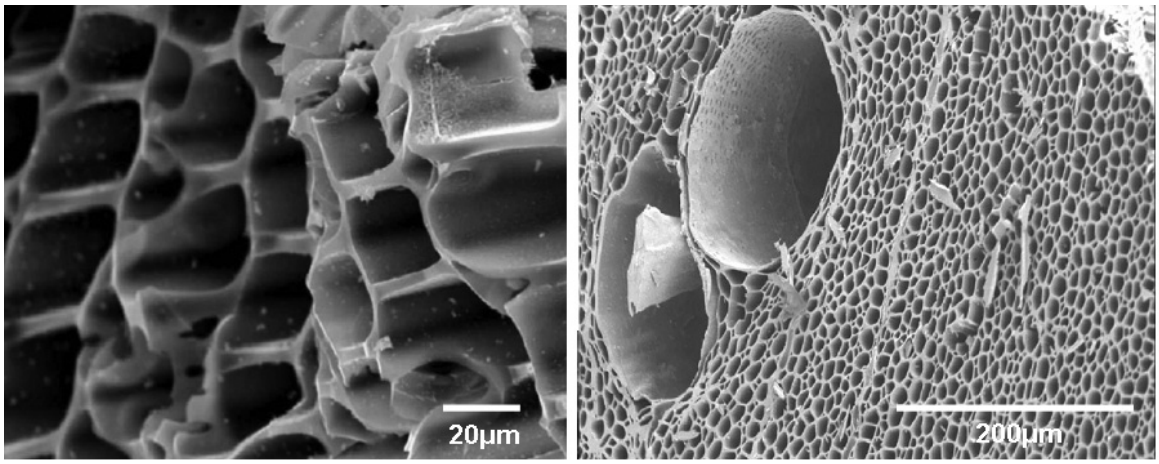


Figure 7: *Macrostructure of PyOM, the nested structure results from the loss of the cell content while only the cell walls remain (Lehmann, 2007).*

Santos et al., 2012; Singh et al., 2012b). The chemical structure and composition of PyOM depends strongly on the parent material, the pyrolysis temperature, with higher temperature and woody feedstock delivering PyOM characterized by a lower H:C (Hammes et al., 2008), an index of aromaticity (Kuhlbusch et al., 1995). As pyrolysis temperature increase the feedstock, originally composed mostly of cellulose, lignin, and pectin is altered and the relative content of phenols, furan, and aromatic-C increases.
PyOM is generally characterized by a higher C content compared to the feedstock due to condensation during the pyrolysis process. Pyrolysis process generally decreases the C:N ratio of PyOM compared original material (Almendros et al., 2003; Knicker, 2007; Hammes et al., 2006) because thermal labile compounds like O-alkyl and carbonyl decreases, while compounds containing N, like amides, have a higher thermal resistance and during pyrolysis they are transformed into new compounds containing N like pyrroles and pyridine (Almendros et al., 2003). Although many uncertainties exist on the mean residence time of PyOM and its drivers, there is a general agreement that PyOM has a higher residence time compared to non-pyrolysed organic matter (Zimmerman and Gao, 2013). A recent meta-analysis from Singh et al. (2012b) estimated PyOM mean residence time to be in the order of magnitude of centuries, although a high variability exists, due to edaphic, environmental, and PyOM characteristics. In fact the category of PyOM is quite broad and contains compounds that have very different origin, like PyOM derived from natural fires (charcoal) and the PyOM derived from artificial pyrolysis of agricultural residues, i.e. biochar. A brief description of the biochar technique is given in the paragraphs below.

1.6 PyOM as a tool to store C and increase soil fertility: biochar

The high resistance of PyOM to decomposition has raised the idea of using it to store C in the soil in the form of thermally degraded organic matter, using a technique called ”biochar technique”. The biochar technique consists in pyrolysing (heating under anoxic condition) organic wastes to obtain heat and a specific char (named biochar) as by-product

(Lehmann, 2007). Due to its slow decomposition rate biochar can contribute to reduce CO_2 efflux by adding a form of C with a slower decomposition rate compared to non-pyrolysed organic matter (Figure 8). Moreover, pyrolysis is an exo-thermal process and therefore the

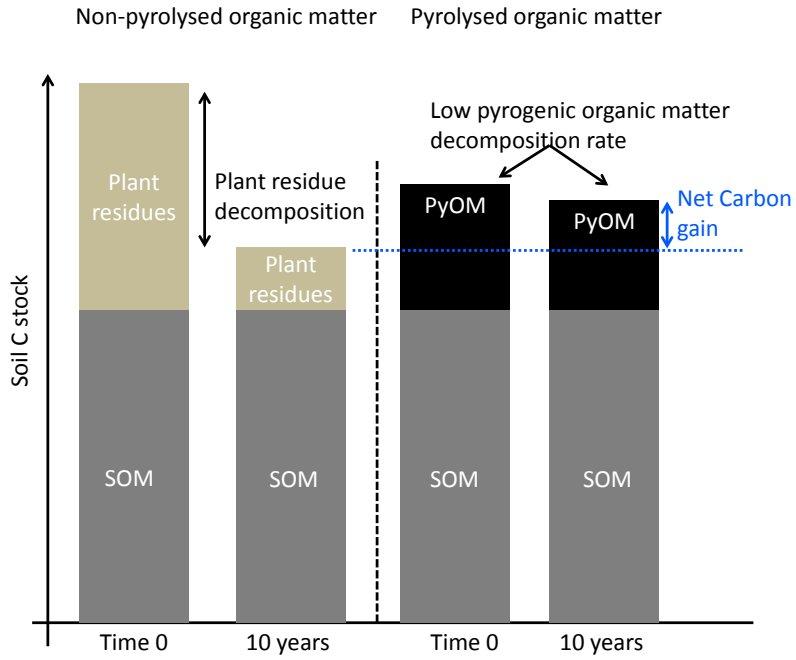


Figure 8: *C storing mechanism through the use of pyrolysed organic matter. The net C gain after a number of years (here ten years, as an example) derives from the lower PyOM-C mineralization rate compared to non-pyrolysed material. This graph is only conceptual and was not produced using real data. Therefore no unit is reported on the y axis.*

production of biochar produces heat, thus saving additional amounts of C, in the form of saved fossil fuels consumption. Also, the application of biochar to the soil is known to improve crop productivity because by increasing soil pH and improving water retention (Jeffery et al., 2011). However the possible agronomic benefits derived from the use of biochar will not be part of the present thesis that focuses on the stabilization of organic matter.

Nevertheless the PyOM addition may also raise concerns mainly due to the high content in polycyclic aromatic hydrocarbon (Hilber et al., 2012). Also it has been suggested that the addition of biochar to the soil may increase the mineralization of native soil organic matter (priming effect Wardle et al. (2008), i.e. it may induce a C-leakage, which is lost from the native organic matter. This latter aspect in particular has been addressed in the present thesis (see section 1.9).

1.7 Interaction between PyOM and N cycle

Influence of N input on PyOM decomposition

As outlined in the section 1.2 N inputs to the terrestrial system are expected to increase sharply in the future. Nevertheless very little is known on the impact of increased N in-

put on soil organic matter mineralization, even though there are evidences that increased N availability will influence soil organic mineralization. Janssens et al. (2010) compiled a meta-analysis of results from 36 N manipulation studies and observed that N addition decreased soil respiration. They proposes three mechanisms to explain such decrease: (i) a shift in the soil microorganisms community toward microorganisms that are more C efficient and less N limited (ii) a reduction in the activity of phenol-oxidase, an enzyme responsible for the cleavage of aromatic compounds like lignin (Sinsabaugh, 2010), and (iii) an increase in the NH_3 following from the higher N availability that acts as a catalyst for the polymerization of phenols resulting in the formation of compounds more resistant to microbial decomposition(Lindbeck and Young 1965, in Nömmik and Vahtras 1982).
Nitrogen is likely to affect particularly the decomposition of fresh organic matter that represents an important fraction of soil respiration. There is a vivid scientific debate on the effect of N addition on fresh organic matter, with two opposing theories: the *N mining theory* and the *stoichiometric theory* (Knicker, 2011). The first one predicts that litter characterized by a high C:N will increase the decomposition of the labile fraction of litter (typically less rich in N) to obtain the energy necessary to "mine" for N the recalcitrant fraction of the litter (richer in N), resulting in an increase of soil respiration, at least on the short term (Craine et al. (2007). On the opposite the stoichiometric theory starts from the assumption that soil microorganisms has a fixed elemental ratio (Cleveland and Liptzin, 2007), characterised by a C:N lower than the litter input, and therefore low content of N are a factor limiting their activity. The two theories obviously bring to different decomposition patterns.
So far it is not known if any of the two theories applies to PyOM decomposition. In fact PyOM although is rich in C this is in a form poorly available to microorganisms, while little is known on the availability of PyOM-N (also called black nitrogen).
The present thesis deals with the effect of N addition on PyOM mineralization rate, a topic that has been poorly investigated. Santos et al. (2012) incubated PyOM in a granitic soil at two contrasting N level and did not find an effect of N addition on PyOM mineralization. Nevertheless it is possible that the N addition may play a role in the activity of the phenol-oxidase which in turn may play a role in the cleavage of PyOM, since this is an aromatic compound.

Effect of PyOM on N availability in soil

While not much is known on the effect of N inputs on PyOM decomposition, the effect of PyOM addition on mineral N availability has been deeply investigated for its important agronomic implications. Results are not univocal, it was found that PyOM increases gross N mineralization (Nelissen et al., 2012), net nitrification (DeLuca et al., 2002; DeLuca and Sala, 2006; Steiner et al., 2008; Wang et al., 2012) and gross nitrification (DeLuca and Sala, 2006; Ball et al., 2010; Nelissen et al., 2012). However, it was also found that PyOM induced N net immobilization (Kolb et al., 2009; Novak et al., 2010; Bruun et al., 2012) or did not alter the N cycle at all (Zavalloni et al., 2011; Zhang et al., 2011). Several reasons can explain the influence of PyOM on the state of N in the soil: (i) it may increase soil

pH and thus creates more favorable conditions for nitrifiers in soil (Ste-Marie and Pare, 1999; Brady and Weil, 2007) (ii) it can adsorb phenols that are inhibitors of nitrification (DeLuca and Sala, 2006; Ball et al., 2010),(iii) it can cause N immobilization due to the high C:N ratio of PyOM, (iv) it can increase native organic matter mineralization (i.e. positive priming effect). In the present study the effect of the addition of a ryegrass-derived PyOM having a low C:N ratio on mineral N content in soil, was investigated with a special regard to coupling N and C cycle.

1.8 PyOM stability

When producing the first estimates on PyOM global production Seiler and Crutzen (1980) assumed that PyOM was not decomposing or that its decomposition rate was so slow to be almost negligible. However as studies and technique to isolate, identify and trace PyOM decomposition progressed, PyOM mean residence time in soil was found to be more variable than expected, ranging from decades to millennia. Pessenda et al. (2001) found that charcoal in the soil had a radiocarbon age of more than 1000 years. Studies based on soil chronosequences (Hammes et al., 2008; Schneider et al., 2011; Nguyen et al., 2008) found that PyOM has a residence time ranging in the centennial time scale. While several litter decomposition studies employing C isotopes revealed a turnover time ranging of decadal to centennial time scale Hamer et al. (2004); Hilscher et al. (2009); Major et al. (2010); Kuzyakov et al. (2009). A meta-analysis from Singh et al. (2012b) revealed that grouping together 13 studies on PyOM disappearance from the soil PyOM had a mean residence time of approximately 300 years. They also observed that grass-derived PyOM, and high temperature pyrolysis generally delivers more resistant PyOM. Nevertheless the analysis showed a great variability in the results and a lack of field studies, with only four studies carried out so far, three of them in the tropics and one in the Russian steppe, with a unexpected lack of studies in the temperate and boreal regions, where fire frequency is expected to increase (Figure 6).

Losses of PyOM by leaching and erosion

There are indirect evidences that PyOM translocation to water bodies occurs, (section 1.4). PyOM is present in large quantities in the marine (Dittmar, 2008; Dittmar et al., 2012) and in the riverine environment (Hockaday et al., 2006; Masiello, 2004; Guggenberger et al., 2008). PyOM transfer from soil to water bodies occurs mostly by leaching and erosion. While it is likely that part of PyOM is rapidly transported by erosion due to light nature of PyOM (Rumpel et al., 2006) very little is known on the quantity of PyOM that can be lost by leaching (Zimmerman and Gao, 2013).
There are contrasting evidences that PyOM can be lost by translocation to belowground. Major et al. (2010), found that losses as DOC were less than 1% of losses as CO_2, and hypothesized that erosion losses accounted up to 25-30% based on PyOM-C recovery after two years. In a column incubation experiment, Hilscher and Knicker (2011) found a fast relocation of PyOM-C from 0-2 cm to 2-5 cm below the topsoil which they attribute to a

water soluble fraction of C present in the PyOM. Also Leifeld et al. (2007) observed an important translocation of PyOM (21-69 %) from the ploughed portion of a drained peatland to below 30 cm. . They hypothesized that transport of PyOM occurred in particulate or soluble form downwards the profile.
Despite findings on vertical transport of PyOM, the fraction of soluble C present in fresh PyOM seems to be relatively small, ranging between 0.03% (Jones et al., 2011) and 0.2% (Abiven et al., 2011). Zimmerman and Gao (2013) found results in the same range, moreover they observed that increasing pyrolysis temperature from 250 °C to 650 °C decreased PyOM content of soluble C. Moreover Abiven et al. (2011) observed that the soluble fraction increased in PyOM aged in the field, probably as a result of PyOM oxidation.

1.9 PyOM induced priming effect on soil organic carbon mineralization

Thanks to its inherent recalcitrance to decomposition PyOM is an ideal candidate to store C in the soil (Lehmann, 2007; Woolf et al., 2010), however several authors pointed out that the addition of PyOM to the soil, could alter the mineralization rate of non-PyOM C pools, and thus affect its efficiency as a C-sink. Wardle et al. (2008) in a ten years litter bag experiment observed that mixing PyOM and litter resulted in greater C losses than the sum of the two substrates alone. This implies that the addition of PyOM to the soil to store C will induce C-leakages from other C-pools, and therefore reducing the efficiency of PyOM as a tool to store C.
The change in the mineralization rate of non-PyOM-C induced by PyOM will be called priming effect in the present thesis. There is no general agreement on the direction of PyOM induced priming effect. In fact PyOM was found to either induce a positive priming effect, i.e. increasing losses of native organic matter (Wardle et al., 2008; Novak et al., 2010; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011), a negative priming effect (Liang et al., 2010; Cross and Sohi, 2011; Jones et al., 2011), i.e. increasing native organic C stabilization or no priming effect (Kuzyakov et al., 2009; Abiven and Andreoli, 2010; Cross and Sohi, 2011; Santos et al., 2012). Several mechanisms have been proposed as possible explanation for priming effect. It is likely that the change in mineralization of non-PyOM-C in PyOM amended soils is the net result of several mechanisms occurring together. These can be grouped between the one that are expected to induce a positive priming effect and mechanisms expected to induce a negative priming effect.
Among the one inducing positive priming Wardle et al. (2008) proposed the *foci hypothesis* suggesting that PyOM porosity offers to microorganisms protection from predators and desiccation. This hypotheses is supported by the findings from Pietikäinen et al. (2000), who reported that PyOM can actively host a microbial community. The *co-metabolic* hypothesis, proposes that PyOM contains a labile fraction that is readily available to microorganisms, and act a source of energy that triggers their growth (Fabbri et al., 2012; Singh et al., 2012a). Also the change in pH following from PyOM addition to soil (PyOM is generally rich in ashes and carbonates) may induce a flush of organic matter mineralization in the short term (Luo et al., 2011; Badalucco et al., 1992) or to alter the enzymatic activity, and thus to influence soil organic matter decomposition. Among the mechanisms proposed

to justify negative priming effect the physical *protection hypotheses* the physical protection hypothesis, suggests that PyOM promotes physical protection of soil organic matter by either sorption of dissolved organic carbon or by promoting soil aggregation (Kimetu and Lehmann, 2010). The occurrence of these different mechanisms is not mutually exclusive, and the prevalence of one mechanism over the other is likely to determine the direction of priming effect at a given time.
It was observed that priming effect may change direction over time. Zimmerman et al. (2011) observed in the first 90 days a positive priming effect, while negative priming effect appeared at a later stage. Woolf and Lehmann (2012) showed that priming effect can dramatically affect the efficiency of PyOM as a C-sink. They modified the RothC model (Jenkinson and Rayner, 1977), to include the effect that PyOM could play on mineralization of freshly added soil organic C. Suggesting that PyOM on one side would increase the mineralization of the labile fraction of the freshly added organic matter and on the other side it would adsorb part of the fresh organic matter, transferring it from the labile fraction to the stable fraction. They hypothesize that over a time frame of one year C protection mechanisms would prevail and PyOM would induce a negative priming effect. Projecting the impact of priming effect induced by yearly additions of PyOM the result would be a net increase of non-PyOM soil organic C of 50%. Nevertheless a long term projection of the impact of priming may suffer from our poor understanding of the underneath mechanisms. Part of the present thesis (particularly Manuscript II and III) is dedicated to the investigation of the processes that can be responsible for priming effect induced by PyOM.

1.10 Microorganisms and enzymatic activity as a proxy for PyOM and soil organic C decomposition

Paterson et al. (2009) defined microorganisms as the eye of the needle through which organic matter has to pass to be mineralized as CO_2. In fact although microbial biomass-C constitutes about 2% of soil organic carbon, soil microorganisms are driving the dynamics of C and N in soil. The addition of fresh input to the soil, like litter, or organic amendments, is generally supposed to alter microbial structure, mostly by supplying an energy source (the C) and nutrients (the N). Contradictory results on the effect of PyOM on microbial biomass were observed: Steinbeiss et al. (2009) observed a reduction in soil microbial biomass, while in other cases an increase was observed (Bruun et al., 2012; Kolb et al., 2009; Steiner et al., 2008; Liang et al., 2010), also no effect was observed Santos et al. (2012); Bruun et al. (2008). Kuzyakov et al. (2009) showed that incorporation of PyOM in microbial biomass is generally low. This may be partially explained by the low availability of PyOM, but also by the relatively low efficiency of microorganisms feeding on PyOM.
While microorganisms are the engine of most of transformation of organic matter in soil, enzymes are fundamental to promote cleavage of molecules, and several authors observed that the addition of several substrates may influence the soil microbial community and the enzyme production and activity (Nannipieri et al., 2012). Very little is known on PyOM effect on enzyme activity, also due to the difficulty in differentiating between the influence of PyOM on soil microorganisms and eventual artifact due to adsorption of enzyme assay

on PyOM surface Bailey et al. (2011).

2 Research questions

Given the framework described above the present thesis is aimed to answer the following research questions: (i) What is the PyOM decomposition rate in the field? (ii) Which is the relative contribution of DOC to losses of fresh PyOM? (iii)What is the effect of pyrolysis on decomposition rate? (iv) Does PyOM prime the decomposition of non-PyOM C pools? (v) Does N affect PyOM decomposition? (vi) Does PyOM affect N availability? (vii) Does PyOM affect microbial biomass?

3 Experimental approach

To answer the above mentioned research questions three independent studies were conducted: (i) a field study aimed to quantify C losses from PyOM in a temperate forest (Manuscript I and IV), (ii) an incubation experiment to investigate the interaction between PyOM and the native organic carbon, where C and N mineralization dynamics were observed over time and (iii) a meta-analysis on the PyOM induced priming effect to determine which factors drives its size and direction, and to speculate on responsible mechanisms. An overall picture of the points addressed in the present thesis is provided in Figure 9, while a comprehensive table of the different methods employed in the studies is presented in Table 1.

3.1 The use of stable isotopes to trace C and N fluxes in decomposition studies

Isotopes are variants of the same element that differ in the mass. Therefore they share the same electronic structures but differ in the atomic mass. In ecological studies isotopes have been widely used to trace transformations of elements throughout their biogeochemical cycles (Glaser et al., 2013). Under the assumption that the process investigated, e.g. mineralization or leaching, do not discriminate between molecules containing different proportions of the two isotopes, it is possible to derive the relative contribution of two sources of known isotope composition to a pool or a flux. In the present study we used isotopes of C and N, two of the main constituents of organic molecules.

The use of ^{13}C to measure organic matter decomposition

Under natural conditions approximately 99% of C atoms contain 12 neutrons (^{12}C). However it is possible to produce plants artificially enriched in atoms of C containing 13 neutrons (^{13}C) by growing them under an atmosphere constantly enriched in ^{13}C (continuous labelling technique) or sporadically enriched in ^{13}C (pulse labelling technique). In the

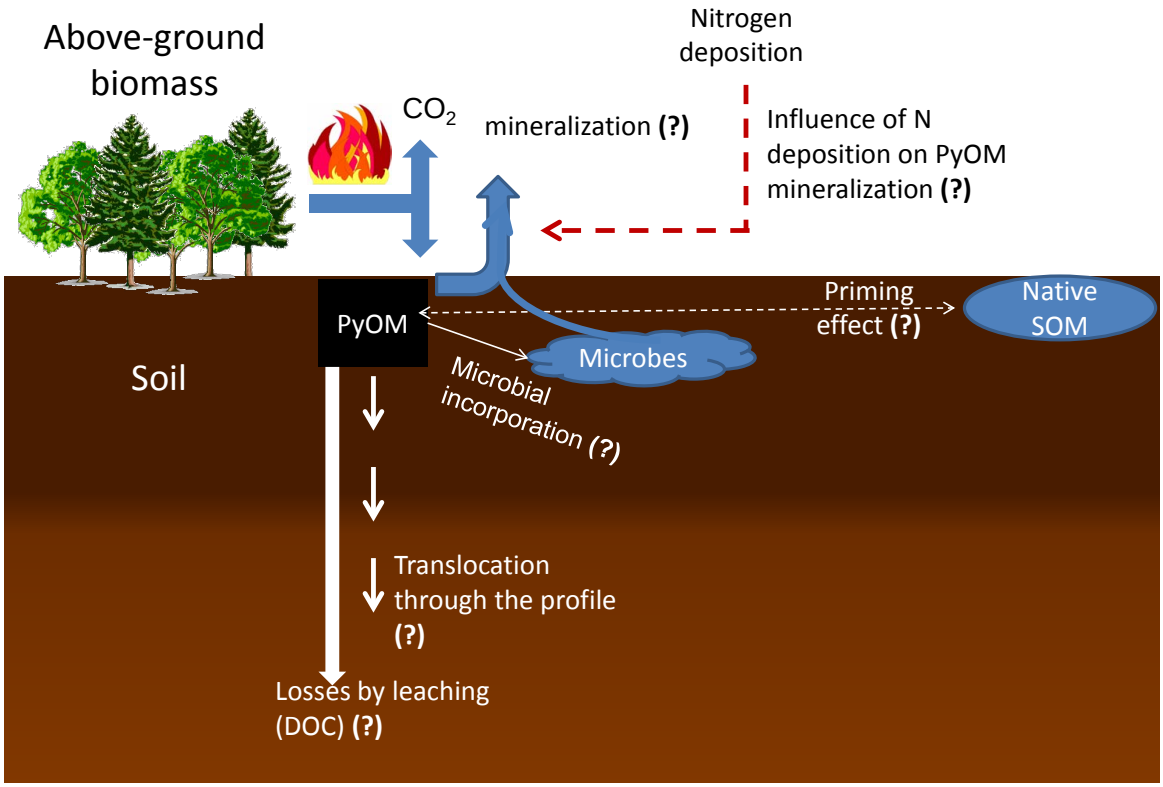


Figure 9: *Description of the PyOM cycle, continuous lines represent the size of the fluxes addressed in the present thesis, dashed lines represent the influence on fluxes. Question marks represent the research questions addressed in the present thesis.*

present thesis both the techniques have been adopted to produce artificially labeled material, the pulse labelling to produce labeled pinewood (Manuscript I and IV) and the continuous labelling to produce ryegrass (Manuscript II). The characteristics of the two substrates used in the present study are presented in Table 2. The PyOM artificially enriched in ^{13}C was used to trace the transformation of C from PyOM to CO_2, DOC, and microbial-C.
A two-pool mixed model was applied (Formula 1) to partition a flux or a pool between two sources of known isotopic composition:

$$f = 1 - (\delta^{13}C_{mix} - \delta^{13}C_{organic\,input})/(\delta^{13}C_{control} - \delta^{13}C_{organic\,input}) \quad (1)$$

Where f = fraction of C derived from the organic input, $\delta^{13}C_{mix}$ indicates the isotopic ratio of C flux or pool to be partitioned, $\delta^{13}C_{control}$ is the isotopic ratio of control soil, and $\delta^{13}C_{organic\,input}$ the isotopic ratio of the added substrate.
In the field experiment (Manuscript I) we used the Keeling plot approach (Keeling, 1958) to estimate the C signature of CO_2 in the soil. The application of this methodology is necessary to estimate the isotopic signature of CO_2 in the soil, since the $\delta^{13}CO_2$ measured during soil respiration is a mixture of atmospheric CO_2 and CO_2 derived from the soil.

Measured Variable	*Method*
Manuscript I and IV (Field experiment)	
Soil respiration	Infrared gas analyser
$^{13}CO_2$	Keeling plot
DOC	Suction plate
PyOM decomposition	Partition of CO_2 and DOC fluxes using isotopes (Manuscript I)
	^{13}C recovery (Manuscript IV)
PyOM quality	BPCA
PyOM incorporation in microbial biomass	Fumigation and isotopic signature of fumigated biomass
PyOM translocation along the profile	^{13}C recovery
PyOM incorporation in soil fractions	Soil density fractionation
Manuscript II (Incubation experiment)	
Soil respiration	NaOH trap
PyOM decomposition	Losses as CO_2
Microbial biomass	Fumigation and isotopic signature of fumigated biomass
Gross N mineralization	^{15}N isotope dilution technique
Net N mineralization	Extraction with 1M KCl
Phenol-oxidase	L-dopa assay method
Protease	Caseine assay method
Manuscript III (Literature data meta-analysis)	
Priming effect size and direction	Weighted mean
Relation between PE and PyOM and soil characteristic	Weighted linear model (boot-strapp)

Table 1: *List of the methodologies applied in the different studies.*

The ^{15}N isotopic dilution technique

The isotopes of N can be used to measure gross N fluxes in the soil. In the present thesis N isotopes were used to measure gross N mineralization in the soil containing PyOM by using the ^{15}N isotopic dilution technique (Khirkham and Bartholomew 1954).
While the classical methods of investigation of N fluxes in the soil consists in the measurement of fluctuation of N in the NH_4^+ and NO_3^- pool, the ^{15}N pool dilution technique

	C	N	H	O	^{13}C	^{15}N
	weight %				atom %	
Pinewood	49.9	0.43	6.6	41.1	2.05	4.3
Pinewood-derived PyOM	79.9	0.71	3.43	14.4	2.03	4.2
Ryegrass-derived PyOM	34.4	3.69	2.02	27	4.33	0.37

Table 2: *Chemical and isotopic characteristics of the raw material and PyOM used in the two studies.*

allows the measurement of the gross fluxes of N. In the present study gross mineralization, the flux of NH_4^+ from the organic N pool to the NH_4^+ pool was measured by adding a known quantity of NH_4^+ enriched in ^{15}N to the soil which contains NH_4^+ containing ^{15}N at natural abundance levels. This is done by monitoring the decrease in $^{15}NH_4^+$ (from here the name of isotope dilution), due to the release of NH_4^+ from the mineralization of organic matter, it is possible to estimate the gross N mineralization.

3.2 Models to predict PyOM decomposition

Several publications have been recently released on PyOM stability, most of them apply laboratory incubations to study PyOM decomposition, as reviewed by Zimmerman and Gao (2013) and Singh et al. (2012b). Since most of the studies on PyOM decomposition have been carried for relatively short period of time, several decomposition models have been fitted to the data, in order to: (i) predict the stability over time and, (ii) compare the stability of PyOM measured over different time scales. Zimmerman and Gao (2013) reviewed the different models that have been applied to PyOM decomposition.
The simplest and most often applied model is the exponential (first order) decay model. This assumes that the rate of PyOM decomposition is a constant fraction of the remaining. The quantity of initial PyOM remaining in the soil according to this model is described by the equation 2:

$$PyOM_t = 100 \cdot e^{-k \cdot t} \tag{2}$$

where the $PyOM_t$ is the PyOM remaining at time t (expressed as % of initial), t is the time, and k is the decomposition rate constant expressed as time^{-1}. The main limitation of the single pool decomposition model is that the turnover rates of PyOM estimated using this equation is strongly time dependent therefore short term experiments typically deliver higher decomposition rates (Zimmerman and Gao, 2013), as presented in Figure 10. This is due to the fact that PyOM is an heterogenous material and is blend of compounds having different decomposition rates.
The two-compartment model is a modification of the single pool (equation 2) that tries to take into account the heterogeneity of compounds present in PyOM. This model assumes that PyOM compounds can be classified in two categories having different decomposition rate and that no exchange between the pools is possible. This model although it represents a simplification of the heterogeneity of molecules contained in PyOM, it is a first approach towards heterogeneity in PyOM and partially solves the problem of the influence of the

duration of the experiment and on PyOM turnover time (Figure 10). Two-compartment

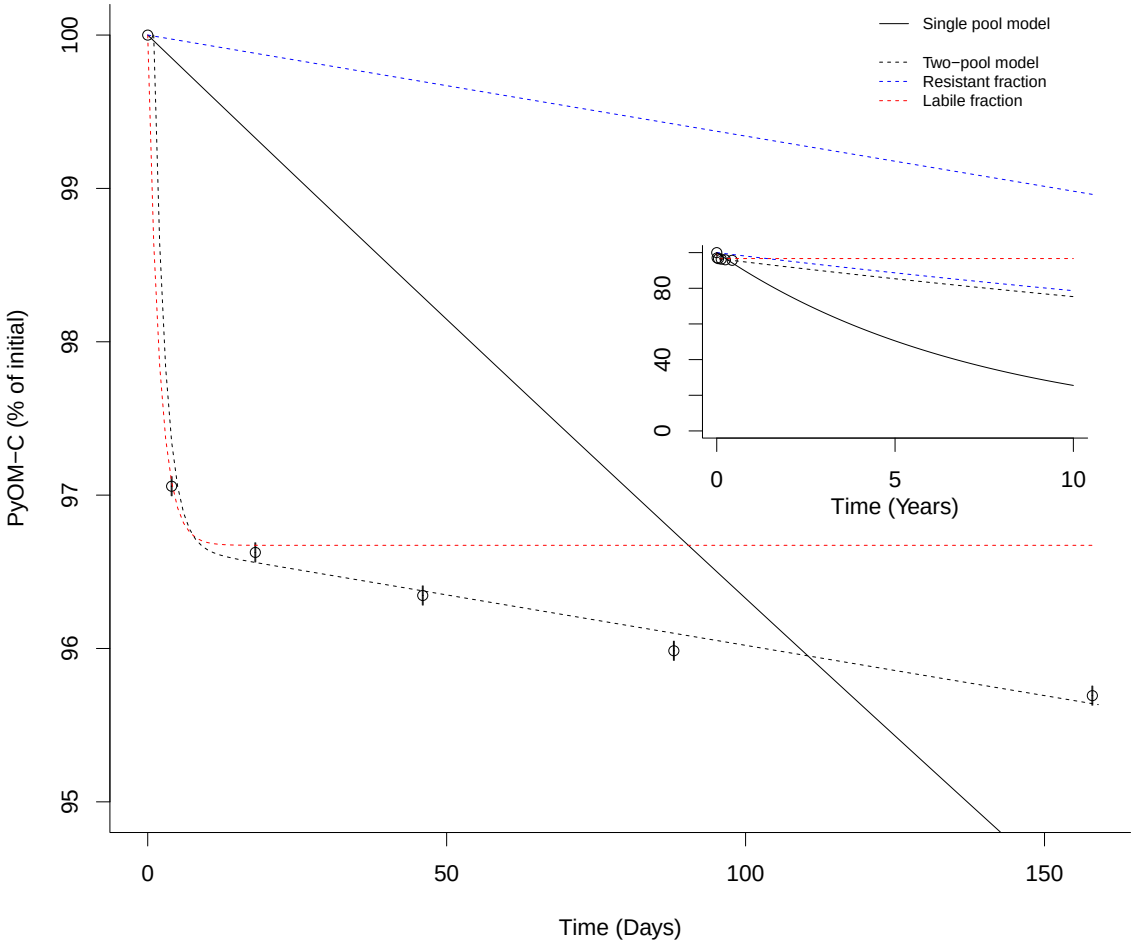


Figure 10: *Comparison of the single pool decay model with the double pool decay model. The single pool has a residual sum-of-squares of 173, while the double pool has a residual sum-of-squares of 1.5. Also the decomposition of the labile and resistant fraction are represented separately. The subplot shows the predicted PyOM-C remaining over a longer time frame of ten years. The two models predict very different quantities of remaining C. The single pool decomposition model is highly influenced by the initial fast decomposition rate (data from Manuscript II).*

models are described by equation 3:

$$PyOM_t = 100 \cdot (f_{labile} \cdot e^{-k_l \cdot t} + (1 - f_{labile}) \cdot e^{-k_r \cdot t}) \quad (3)$$

where the $PyOM_t$ is the PyOM remaining at time t (expressed as % of initial), f_{labile} is the labile fraction of PyOM, and k_l and k_r are the decomposition rate constants of the labile and resistant pool, respectively. These two models are the most widely applied, but also other models have been used sporadically to describe PyOM decomposition: logarithmic model (Bai et al., 2013), a three pool model, with two decomposable and a non-decomposable pool (Farrell et al., 2013), power function model (Zimmerman and Gao, 2013), and Arrhenius model Zimmermann et al. (2012). In the present thesis we used the single (Manuscript I) and two pool decomposition model(Manuscript II), depending on model fitness to the data.

3.3 Field experiment (Manuscript I and IV)

A manipulation field experiment on PyOM decomposition was conducted between October 2009 and October 2010. The study site was located in the Laegeren forest, near Baden (Switzerland), in a mixed forest of beech and spruce. The objectives of this study were: (i) evaluate the efficiency of pyrolysis to produce substrates having long mean residence time (ii) quantify the relative contribution of leaching and mineralization processes to PyOM loss from the soil, (iii) measure the impact of N input on PyoM decomposition, (iv) observe the effect of PyOM addition on native soil organic matter mineralization, (v) measure the incorporation of PyOM C and N in the microbial biomass,(vi) investigate changes in PyOM chemistry over one year in the field, (vii) measure PyOM translocation along the profile and (viii) measure the incorporation of PyOM into the different soil fraction. The experimental setup consisted of mesocosms (15 cm long PVC cylinders) inserted into the soil where pyrolysed and non-pyrolysed pinewood artificially labeled with ^{13}C was added to the first 2 cm of the soil inside the mesocosms (Figure 11). Roots and growing grasses were removed from the mesocosms to eliminate the autotrophic portion of soil respiration. To estimate the impact of pyrolysis on the stability of organic matter we also non-pyrolysed organic matter was added to the soil. On a monthly basis mineral N dissolved in water was added to half of the mesocosms to simulate mineral N deposition from the atmosphere (60 kg N ha^{-1} $year^{-1}$).

To answer research questions i-v we followed for one year the losses of PyOM-C from the soil occurring as CO_2 and DOC, these data are reported in Manuscript I. To answer research questions vi-ix part of the mesocosms installed were extracted after ten months. The ^{13}C recovery in soil at 5, 10, and 15 cm depth and incorporation in microbial biomass, and benzene polycarboxylic acids (BPCA), a molecular marker specific to PyOM that gives information on the condensation of PyOM molecules, were measured. These data are reported in Manuscript IV.

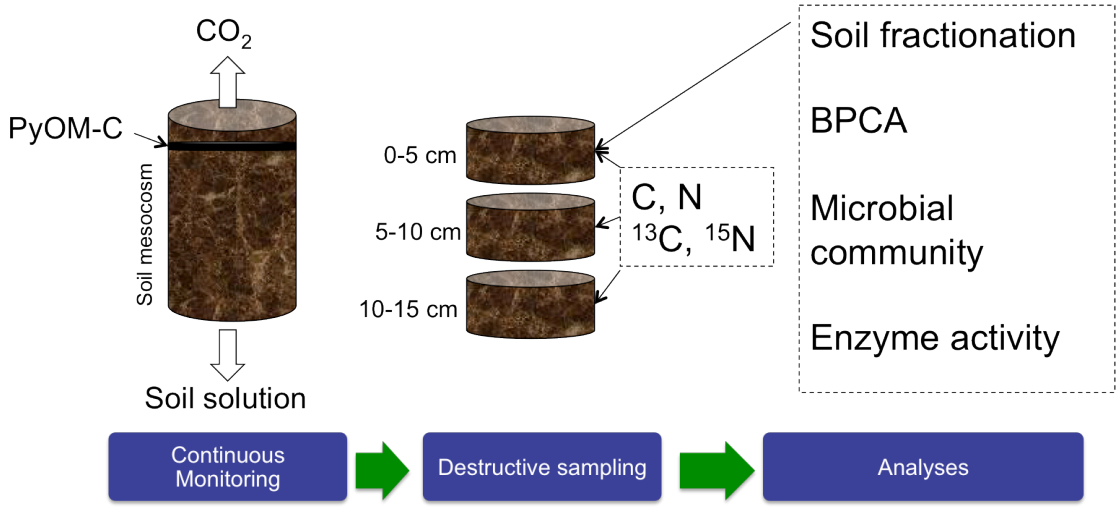


Figure 11: *Experimental design of the experiment in Laegeren (Singh, 2013).*

3.4 Incubation experiments (Manuscript II)

Following the field experiment in Laegeren we performed an incubation experiment aimed to elucidate the mechanisms of interaction between PyOM, native soil organic matter and the addition of mineral N to the soil. This study particularly focuses on the mechanisms regulating C and N fluxes from the soil, with a special regard to changes in soil pH and enzymes activity. The study was carried out in the same soil as the field experiment, but using a different PyOM, because we used the ^{15}N isotopic dilution technique, therefore using the same material as in the first experiment (labeled with ^{15}N) would have introduced an additional source of labeled N, leaving unresolved the equation for the gross N mineralization. We incubated a mix of soil and ^{13}C labeled rye-grass derived PyOM (+4.3 atom %, see section 3.1), at a rate of 1.3 weight % for 5 months. We determined PyOM and native soil organic C mineralization, NH_4^+ and NO_3^- content in the soil, gross N mineralization, phenol-oxidase and protease activities, microbial biomass, throughout the incubation experiment and incorporation of PyOM in microbial biomass at the end of the experiment. The cumulative CO_2 efflux between five sampling dates was measured using NaOH traps. CO_2 trapped in the form of sodium carbonate was then precipited using BaCl. Gross N mineralization was measured using the isotopic dilution technique, briefly five μg N-NH_4^+ g^{-1} soil (^{15}N 3 atom %) were added to the soil and the ^{15}N and NH_4^+ content was measured at regular interval for three days. Microbial biomass was measured using fumigation extraction method, i.e. by lysing the cell with choloroform and measuring C in KCl extract before and after fumigation. Finally enzymatic activity of phenol-oxidase and protease was measured over time, using respectively L-dopa and casein as assay.

3.5 Manuscript III: Meta-analysis

As contrasting results are reported in literature and in the two previously described experiments we conducted a meta-analysis on the priming effect induced by PyOM on native soil organic matter. We compiled out of 20 studies a database of 800 cases, where the C fluxes from soils amended with PyOM were measured and partitioned among the possible sources at different time after PyOM addition to the soil. We investigated the relation between priming effect and the following factors: time, PyOM C content, soil C content, soil pH, PyOM-C addition, PyOM parent material, soil texture. We investigated separately the priming effect induced on the fresh organic and on the native soil organic matter.
The main limit of this study resides in the data harmonization, since data were collected from different experiments and therefore the conversion to a standard formulation required some harmonization procedure. For example in field experiments CO_2 fluxes are typically expressed on a spatial scale while in incubations they are typically related to the weight of the soil producing the efflux, therefore we had to assume that only soil until PyOM addition depth was contributing to soil respiration. Also, the definition of the priming effect induced by PyOM on fresh organic matter, can be controversial, in fact in studies where PyOM, soil and non-pyrolysed fresh organic matter are mixed it is necessary to discriminate between three source of C, but the numbers of isotopes used in the studies on PyOM decomposi-

tion, maximum two does not allow the discrimination of the three sources, therefore some assumptions on the source of the C fluxes were necessary (see Manuscript III). To evaluate which factors were related to priming effect we performed a weighted regression, using boot-strapping to overcome the problem of normality distribution in our data.

Section Highlights

Three independent studies using different approaches were conducted to investigate the impact of PyOM on soil C budget and the influence of N on PyOM decomposition:

- **Field Manipulation experiment**: ^{13}C labeled pinewood-derived PyOM was produced and added to a forest topsoil, with and without mineral N addition (+60 Kg N ha^{-1}). The aim of the experiment was to produce the first assessment of PyOM losses in the field using stable isotopes. We traced the losses of PyOM as CO_2 and DOC during one year (Manuscript I) and measured after 10 months changes in PyOM quality (BPCA method), quantity (^{13}C recovery), PyOM translocation along the profile (Manuscript IV), and incorporation in microbial biomass.
- **Incubation experiment**: A mixture of soil and ^{13}C labeled ryegrass-derived PyOM (1.3 weight % PyOM addition rate) was incubated for five months under controlled humidity aneralization, and whether possible N limitation may drive the priming effect. We measured over five months soil respiration and partitioned it between PyOM and native soil organic matter, microbial biomass and PyOM incorporation after five months, NH_4^+ and NO_3^- content, gross N mineralization using the ^{15}N pool dilution approach, protease and phenol-oxidase.d temperature conditions. The aim of the study was to investigate the relation between PyOM and soil organic C and N mineralization.
- **Literature data meta-analysis** We conducted a meta-analysis of literature data on PyOM induced priming effect on native and fresh soil organic matter. We produced a database reporting priming effect and the corresponding soil and PyOM physical and chemical characteristics, and the time after PyOM addition. We estimated the size and direction of priming effect on native and fresh soil organic matter, and the relation between priming effect and related parameters.

4 Results and discussion

4.1 PyOM mineralization

After one year in the field approximately 0.5% of pinewood-derived PyOM-C was mineralized, while cumulative decomposition of non-pyrolysed material was approximately 60 times higher (Figure 12). The incubation study revealed a lower mineralization rate for the ryegrass-derived PyOM, with a decomposition rate of 4% of initial PyOM-C after five

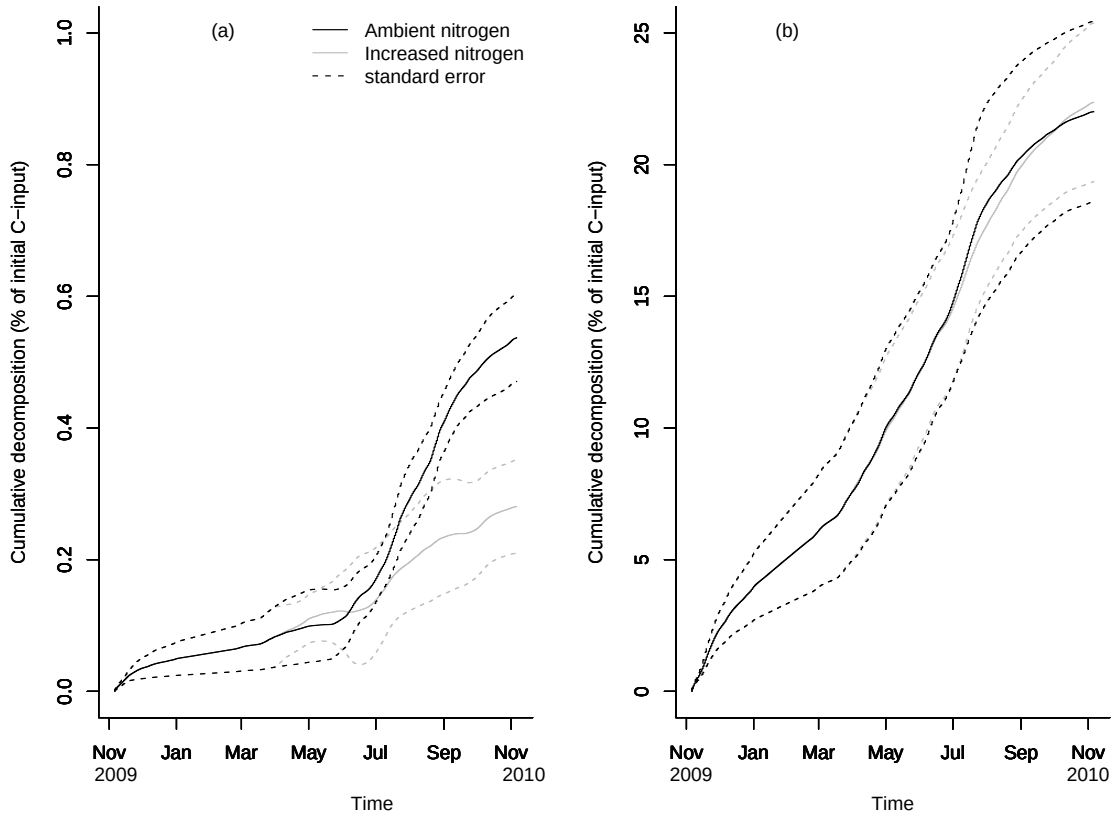


Figure 12: *Figure (a) shows the mineralization rate of PyOM in the field experiment, under ambient (continuous line) and increased N deposition conditions. Figure (b) shows wood mineralization under ambient (continuous line) and increased N deposition conditions (Manuscript I).*

months (Figure 10). Two reasons can explain the difference between the mineralization rates observed in the two studies: (i) the PyOM characteristics and (ii) the experimental approach. The two PyOM differed first of all in the elemental composition. The pinewood-derived PyOM was characterised by a higher C content, a higher C:N and a lower H:C compared to ryegrass-derived one. The latter is an indicator of aromaticity, and a predictor for PyOM stability (Spokas et al., 2009). The PyOM used in the incubation study had a high H:C ratio, indicating that either pyrolysis transformed part of the organic carbon into carbonates or that part of the biomass remained uncharred during the pyrolysis process.
Also the experimental approach may have substantially influenced PyOM decomposition rates. Incubations are in fact known to produce higher decomposition rates than field experiments due to more favourable conditions of constant humidity and temperature. With this regard the present studies confirmed findings from (Singh et al., 2012b) who observed that PyOM losses measured in incubation experiments were significantly higher that the one measured in field experiments. We can rule out that the length of the observation period has played a role, as suggested by Singh et al. (2012a). In fact first the two experiments had a comparable duration and second in the incubation experiment the PyOM decomposition was levelling off already after the first months, therefore we can exclude that we captured

only the initial flush of PyOM decomposition.
In order to predict remaining litter over time first order decomposition models are often used Olson (1963). As this was not the object of the present we can not confirm the goodness of this type of model for PyOM stability on the long term. Nevertheless, we can use them to speculate on the remaining PyOM-C over 100 years (the minimum residence time required by most C offset program). Using this type of model we observed that 50% pinewood-derived PyOM is stable for at least 100 years while only 8% of ryegrass-derived PyOM would remain after 100 years (Manuscript I and II).

4.2 PyOM loss pattern

The field study clearly indicates that the main pathway for the losses of fresh PyOM-C loss was mineralization to CO_2. We found that losses of PyOM as dissolved organic carbon (DOC) were three orders of magnitude lower compared to losses as CO_2 (Manuscript I). Moreover also the translocation of PyOM along the profile was negligible (Manuscript IV). Our findings confirm the little solubility of fresh PyOM (Abiven et al., 2011; Kuzyakov et al., 2009; Jones et al., 2011). To reconcile our results with evidences of substantial quantities of PyOM recovered in the oceans (Dittmar, 2008; Ziolkowski and Druffel, 2010; Dittmar et al., 2012), I hypothesize that losses of PyOM by erosion are the main mechanism for transfer of PyOM from the soil to water bodies. Major et al. (2010), in an experiment similar to ours hypothesized that losses by erosion could account up to 35% of added PyOM-C confirming findings from Rumpel et al. (2006).
Moreover it is likely PyOM becomes more soluble with ageing as a result of increased oxidation (Abiven et al., 2011), therefore it is possible that losses of PyOM from our study will increase substantially in the future. Also, part of PyOM leaching through the soil may be blocked in the soil matrix, since the soil of the study site had a high clay content.

4.3 PyOM and priming effect

We found contrasting results regarding the interaction between PyOM and native soil organic matter: in the field experiment, both the C recovery at the end of the experiment and the CO_2 efflux and partition measurements indicated that PyOM may have primed the losses of native soil organic C (Manuscript I and IV). Nevertheless the priming effect was not statistically significant, most likely due to the high spatial variability and the little number of replicates. The incubation experiment showed a two-phase pattern for priming effect, a strong initial positive priming effect was observed at the beginning of the experiment followed by a phase of negative priming effect that persisted until the end of the experiment. N did not affect the priming effect (Manuscript II, Figure 13). When cumulated over time the mineralization of native soil organic matter was higher in the control than in the PyOM addition treatment, i.e. we observed a cumulative negative priming effect after five months of incubation. The meta-analysis on priming effect showed a similar pattern with positive priming effect on the short term and negative priming effect appearing at a later stage.
Several mechanisms have been proposed to justify priming effect, they are discussed in

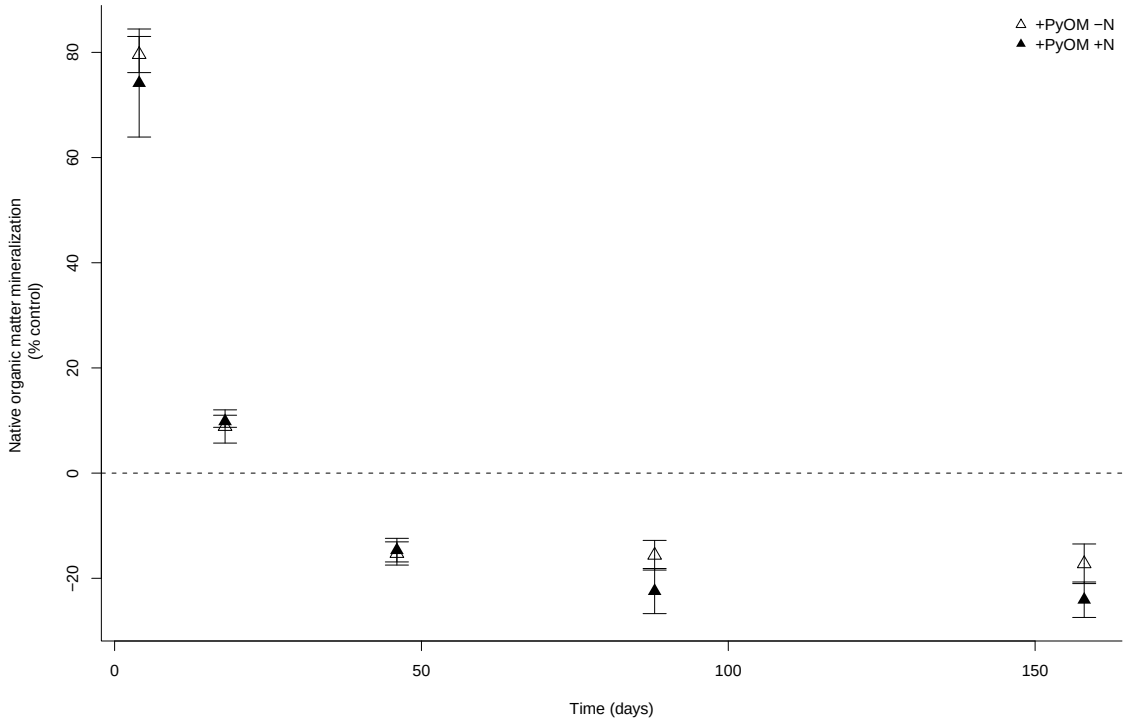


Figure 13: *Priming effect induced by PyOM on native soil organic matter mineralization. Priming effect is expressed as change of the mineralization rate with to control. Empty triangles indicate priming effect in no N addition treatment while full triangles are priming effect in N addition treatment (data from Manuscript II).*

depth in the Manuscript III. I believe that in the incubation experiment the increase in microbial activity can be attributed either to the presence of a labile fraction in the PyOM or the PyOM induced increase of soil pH. The initial positive priming effect caused a depletion of the C pool, and limited the substrate available to microorganisms causing therefore the negative priming effect. Alternatively the negative priming effect may have been caused by the adsorption of native soil organic matter on PyOM (physical protection mechanism). The meta-analysis revealed also that a positive initial priming effect was negatively correlated to PyOM C content, i.e. the PyOM was characterized by a low degree of condensation, was more available to microorganisms and thus acted as a feeding substrate. We hypothesize that negative priming effect is due to the influence of PyOM on soil physical characteristics like soil aggregation (Vasilyeva et al., 2011), i.e. to the increase of physical protection. While in Manuscript I, II and IV we investigated the influence of PyOM on native soil organic carbon, in Manuscript III we also observed the effect of PyOM on fresh organic matter, we found that PyOM induce mostly a negative priming effect on the fresh organic matter, however it was not possible to identify the factors responsible for this, due to the paucity and heterogeneity of literature data on the topic.

4.4 N influence on PyOM mineralization

N addition reduced mineralization of PyOM when measured as CO_2 losses in the field (Manuscript I), however this result was not confirmed by the C recovery ten months after the addition of the PyOM (Manuscript IV, Figure 12). This difference may be due to the low sensitivity of the C recovery method compared to the CO_2 measurement. However in the incubation experiment we did not observe an effect of N addition on PyOM decomposition (Manuscript II). The factors that could explain such different behavior between the two experiments are: (i) the C:N of PyOM that was much higher in the pinewood-derived PyOM (Table 2) and, (ii) the frequency of N addition, in fact in the incubation experiment N was added all at the beginning of the experiment, while in the field study N was added monthly. The C:N of the substrate may alter the impact of N input, in fact the addition of N to substrate with a high C:N may prevent the microorganisms from mining the recalcitrant part of the substrate from mining for N. However in an incubation experiment using the same PyOM of our field experiment Santos et al. (2012) did not find any effect of N on PyOM mineralization. This may suggest that the way N is added to the system may influence its effect on PyOM. In fact it could be that PyOM adsorb the N (Jones et al., 2012), and therefore, when added in one single solution, the effect of PyOM rapidly disappears.

4.5 PyOM influence on N mineralization

In the incubation experiment we observed that PyOM increased gross and net N mineralization rate on the short term (three days). However it was not possible to distinguish whether the increase in mineral N was derived from decomposition of PyOM or from the increased decomposition of native soil organic matter, i.e. was due to priming effect (Manuscript II). Given the good relation between N mineralization and CO_2 efflux (see Figure 14, we believe that the increase in microorganisms activity stimulated by PyOM induced also an increase in mineral N content of the soil. However recent evidences (Knicker et al., 2013) showed that PyOM-N is readily assimilated by plants and microorganisms. Irrespective of the mechanism involved in the increase of N mineralization PyOM followed the rule of the correlation between C:N ratio and N mineralization: substrates characterized by low C:N ratio tend to release N on the short term, confirming findings from Bruun et al. (2012).

4.6 Incorporation of PyOM in microbial biomass

Both field and laboratory incubation experiments highlighted that only a marginal quantity of PyOM was incorporated in the microbial biomass, equivalent to 0.46% and 0.15% of initial PyOM, in the laboratory (Manuscript II) and in the field experiment respectively (Manuscript IV). This confirms that irrespective of the differences in PyOM decomposability of the two PyOM used in the experiment, the availability of PyOM to microorganisms was limited. The low incorporation of PyOM into the microbial biomass suggests that PyOM is probably not their main feeding substrate, at least not after several months when, if present, the labile fraction of PyOM has been decomposed.

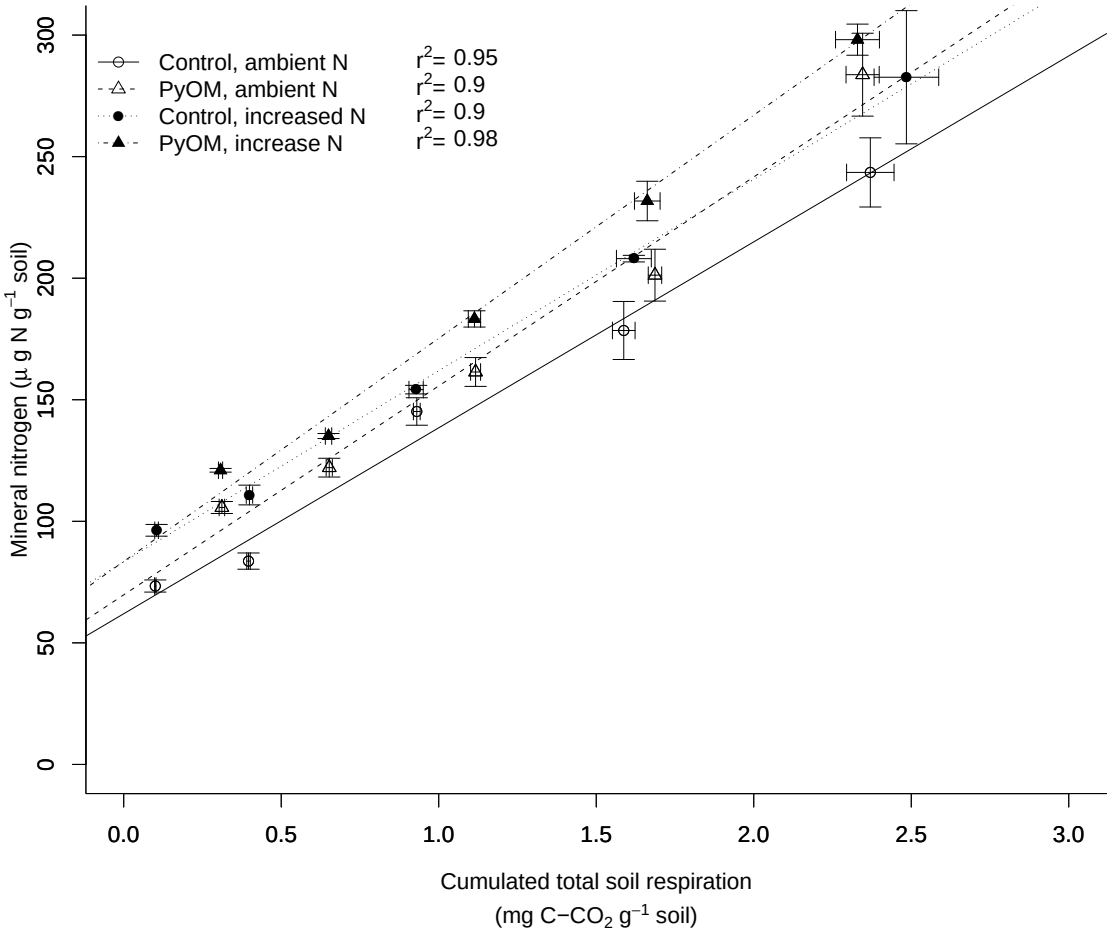


Figure 14: *Correlation between cumulative total CO_2 production (since the addition of PyOM), and soil mineral N content of the different treatment (data from Manuscript II), in the PyOM incubation experiment.*

Section Highlights

In this section the results from the three studies were presented using a synoptic approach. We observed that:

- **PyOM decomposition** of pinewood-derived PyOM was slower than ryegrass-derived PyOM. This may be attributed both to the PyOM characteristics, and to the experimental setup (field experiment vs incubation).
- Both the incubation and the meta-analysis showed that PyOM may induce a double phase **priming effect** with a positive priming effect on the short term followed by a phase of negative priming.
- **N addition** did reduce the decomposition rate of PyOM in the field experiment but not in the incubation experiment. This may be attributed to the different characteristics of the PyOM used (ryegrass derived with low C:N vs. Pinewood derived with high C:N) or to the frequency of N addition (single addition at the beginning of the experiment or monthly addition).
- Ryegrass-derived PyOM increased **gross and net N mineralization** on the short term.
- **Incorporation** of PyOM in microbial biomass was extremely low both after 5 months (ryegrass-derived PyOM incubation) and after one year (pinewood-derived PyOM field experiment).

4.7 Implications for the C cycle

Our results indicate that wood-derived PyOM has a mean residence time ranging in the centennial time scale, when measured in the field. A hundred years is the minimum residence time required by many voluntary C emission reduction schemes to consider it as beneficial in terms of C sequestration. If we adopt this threshold in studies on the C budget of forest fires we can conclude that neglecting the impact of charcoal production may lead to an overestimation of the impact of the fire on C emissions. Nevertheless results from our field studies on the decomposition pattern of PyOM showed that the interaction between environmental conditions like temperature and PyOM decomposition may limit the application of the most commonly used models (like exponential decomposition models) to predict PyOM stability. Therefore I believe that the incorporation of PyOM into C budget is so far mostly limited by: (i) Uncertainty on the PyOM production rate depending on fire conditions (ii) limited knowledge on the applicability of most commonly adopted decomposition models (RothC, Yasso) to soils containing PyOM. The present study also shows that priming effect may significantly impact the C cycle, at least on the short term. While this has for sure implications for the cycling of other elements like N, that are bounded to C and whose mineralization is strictly correlated to the one of C (see manuscript II), less is known on its long term impact as studies are lacking specially in the field. Interestingly my

study did not show a correlation between the rate of PyOM added and the priming effect observed, but rather with the type of PyOM added with PyOM containing a labile fraction inducing more positive priming effect on the short term. When looking at this result from the perspective of the environmental mangers intending using biochar we can recommend that the possible presence of a labile fraction in biochar needs to be taken into account when engineering the production of the biochar material.

5 Conclusion:

This thesis investigated the role of PyOM on the soil C and N dynamics. We showed for the first time in a field experiment in the temperate region, using PyOM artificially enriched in ^{13}C that pyrolysis reduces the decomposition rate of pinewood by a factor of 60. This evidence strongly indicates that the charcoal produced during forest fires needs to be taken into account when producing C budget of fire events. Similarly it indicates that pyrolysis can increase the mean residence time of agricultural residues (*biochar*) in soil and therefore mitigate climate change. However a number of evidences from this work warn us on the factors that may alter the net effect of PyOM on C storage. Particularly it was observed that N may affect PyOM stability, depending on PyOM chemical composition and the frequency of N addition. Also the feedstock material will greatly influence the stability of PyOM; particularly we observed that grass-derived PyOM was less stable than wood-derived PyOM. However a direct comparison of pinewood and ryegrass decomposition rate in the present thesis was probably influenced by the different methodology adopted.
This work suggests that the presence of a labile fraction in PyOM may induce priming effect and increase N mineralization rate as a result of increased microbial activity.
Therefore I believe that treating PyOM as a blend of molecules of various microbial availability, rather than as an homogeneous material, will improve the capacity to predict PyOM impact on the C balance. A first approximation to this approach maybe partitioning PyOM into two pools of different stability.
We also investigated the pathway for PyOM losses and found that losses of fresh PyOM as dissolved organic carbon and translocation through the soil profile were minimal compared to losses as CO_2. This result contributes to resolve the conundrum of the origin of the high quantity of PyOM observed in oceans and rivers. In fact the absence of important losses by leaching points toward erosion as the main factor of PyOM transport to water bodies.
I conclude that when producing the C-budget of soils containing PyOM, next to more traditional factors like feedstock material and pyrolysis temperature also factors like the interactions with non-PyOM and the status of nutrient in the soil need to be taken into account. Particularly the presence of a labile pool in PyOM, and its size may be an important predictor for the capacity of PyOM to serve as a feeding substrate for microorganisms whose activity drives C and N mineralization in the soil.

6 Research Perspectives

Research on PyOM has received an increasing attention over the last ten years. Several books, journal special issues and conferences has been released and organized on this topic. I believe that one of the main challenges in PyOM research for the next future will be producing a comprehensive evaluation of the published experimental works. Based on the current state of research I outline below some of the research topic that I believe will need further investigation in the future:

6.1 Integrating research on biochar PyOM and wildfire derived PyOM

A dualism exists in literature between biochar derived PyOM and fire derived PyOM. I believe that a deeper investigation of the differences among these two types of PyOM would allow the integration of results from studies on PyOM of different origin. In particular I believe that understanding the effect of different oxygen levels during pyrolysis is the key to comprehend the differences between biochar produced under absence of oxygen and fire-derived PyOM produced under local and temporary oxygen limitation.

6.2 Estimating the size of the labile fraction in PyOM

There is a growing body of evidences that PyOM contains a labile fraction that represent the active fraction of PyOM. The size of this fraction is likely to be responsible for many of the short term effect of PyOM, like rapid initial loss of PyOM (the so called Black Carbon paradox), priming effect, and release of nutrients from PyOM and soil organic matter. I believe that the definition of a standardized methodology to rapidly measure or predict the size of the labile fraction in PyOM will improve our capacity to assess the impact of PyOM on soils, particularly in agroecosystems.

6.3 PyOM in water: erosion or leaching of old PyOM

The conundrum on the origin and fate of PyOM in water bodies is not yet fully resolved. Although evidences that erosion can produce significant PyOM losses are increasing, this topic did not receive enough attention probably because of the challenges in measuring eroded PyOM-C. Investigating PyOM-C losses their fate once they have reached water bodies will greatly improve our understanding of the impact of PyOM on the C cycle.

Bibliography

Abiven, S. and Andreoli, R., 2010. Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. Biology and Fertility of Soils 47:111–114.

Abiven, S., Hengartner, P., Schneider, M., Singh, N., and Schmidt, M., 2011. Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. Soil Biology and Biochemistry 43:1615–1617.

Almendros, G., Knicker, H., and González-Vila, F. J., 2003. Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state 13C- and 15N-NMR spectroscopy. Organic Geochemistry 34:1559–1568.

Ascough, P., Bird, M., Francis, S., Thornton, B., a.J. Midwood, a.C. Scott, and Apperley, D., 2011. Variability in oxidative degradation of charcoal: Influence of production conditions and environmental exposure. Geochimica et Cosmochimica Acta 75:2361–2378.

Badalucco, L., Grego, S., Dell'Orco, S., and Nannipieri, P., 1992. Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (Picea abies L.). Biology and Fertility of Soils 14:76–83.

Bai, M., Wilske, B., Buegger, F., Esperschütz, J., Kammann, C. I., Eckhardt, C., Koestler, M., Kraft, P., Bach, M., Frede, H.-G., and Breuer, L., 2013. Degradation kinetics of biochar from pyrolysis and hydrothermal carbonization in temperate soils. Plant and Soil 372:375–387.

Bailey, V. L., Fansler, S. J., Smith, J. L., and Bolton, H., 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biology and Biochemistry 43:296–301.

Ball, P. N., MacKenzie, M. D., DeLuca, T. H., and Holben, W. E., 2010. Wildfire and Charcoal Enhance Nitrification and Ammonium-Oxidizing Bacterial Abundance in Dry Montane Forest Soils. Journal of Environment Quality 39:1243.

Bird, M. I. and Grocke, D. R., 1997. Determination of the abundance and carbon isotope composition of elemental carbon in sediments. Geochimica et Cosmochimica Acta 61:3413–3423.

Bond-Lamberty, B. and Thomson, a., 2010. A global database of soil respiration data. Biogeosciences 7:1915–1926.

Bowman, D. M. J. S., Balch, J. K., Artaxo, P., Bond, W. J., Carlson, J. M., Cochrane, M. a., D'Antonio, C. M., Defries, R. S., Doyle, J. C., Harrison, S. P., Johnston, F. H., Keeley, J. E., Krawchuk, M. a., Kull, C. a., Marston, J. B., Moritz, M. a., Prentice, I. C., Roos, C. I., Scott, A. C., Swetnam, T. W., van der Werf, G. R., and Pyne, S. J., 2009. Fire in the Earth system. Science (New York, N.Y.) 324:481–4.

Brady, N. and Weil, R., 2007. *The Nature and Properties of Soils*. Prentice Hall PTR, Upper Saddle River, New Jersey.

Bruun, E. W., Ambus, P., Egsgaard, H., and Hauggaard-Nielsen, H., 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biology and Biochemistry 46:73–79.

Bruun, S., Jensen, E. S., and Jensen, L. S., 2008. Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. Organic Geochemistry 39:839–845.

Chapin, F. S., Matson, P. A., and Mooney, H. A., editors, 2002. *Principles of terrestrial ecosystem ecology*. Springer.

Chuvieco, E., 1999. Measuring changes in landscape pattern from satellite images: Short-term effects of fire on spatial diversity. International Journal of Remote Sensing 20:2331–2346.

Cleveland, C. C. and Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a Redfield ratio for the microbial biomass? Biogeochemistry 85:235–252.

Craine, J. M., Morrow, C., and Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88:2105–13.

Cross, A. and Sohi, S. P., 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biology and Biochemistry 43:2127–2134.

Crutzen, P. J. and Andreae, M. O., 1990. Biomass burning in the tropics: impact on atmospheric chemistry and biogeochemical cycles. Science 250:1669–78.

De Schrijver, A., Verheyen, K., Mertens, J., Staelens, J., Wuyts, K., and Muys, B., 2008. Nitrogen saturation and net ecosystem production. Nature 451:E1; discussion E3–4.

DeLuca, T., Nilsson, M.-C., and Zackrisson, O., 2002. Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia 133:206–214.

DeLuca, T. H. and Sala, A., 2006. Frequent fire alters nitrogen transformations in ponderosa pine stands of the inland northwest. Ecology 87:2511–22.

Dittmar, T., 2008. The molecular level determination of black carbon in marine dissolved organic matter. Organic Geochemistry 39:396–407.

Dittmar, T., de Rezende, C. E., Manecki, M., Niggemann, J., Coelho Ovalle, A. R., Stubbins, A., and Bernardes, M. C., 2012. Continuous flux of dissolved black carbon from a vanished tropical forest biome. Nature Geoscience 5:618–622.

Fabbri, D., Torri, C., and Spokas, K. a., 2012. Analytical pyrolysis of synthetic chars derived from biomass with potential agronomic application (biochar). Relationships with impacts on microbial carbon dioxide production. Journal of Analytical and Applied Pyrolysis 93:77–84.

Farrell, M., Kuhn, T. K., Macdonald, L. M., Maddern, T. M., Murphy, D. V., Hall, P. a., Singh, B. P., Baumann, K., Krull, E. S., and Baldock, J. a., 2013. Microbial utilisation of biochar-derived carbon. The Science of the Total Environment 465:288–297.

Flannigan, M., Cantin, A. S., de Groot, W. J., Wotton, M., Newbery, A., and Gowman, L. M., 2013. Global wildland fire season severity in the 21st century. Forest Ecology and Management 294:54–61.

Forbes, M. S., Raison, R. J., and Skjemstad, J. O., 2006. Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. The Science of the total environment 370:190–206.

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., and Cosby, B. J., 2003. The Nitrogen Cascade. BioScience 53:341.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., and Vo, C. J., 2004. *Nitrogen cycles : past , present , and future*.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. a., Seitzinger, S. P., and Sutton, M. a., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science (New York, N.Y.) 320:889–92.

Glaser, B., Wiedner, K., and Dippold, M., 2013. Studying the role of biochar using stable isotopic techniques. In N. Ladygina and F. Rineau, editors, *Biochar and soil biota*, chapter 5, pages 134–164. CRC press, Boca Raton.

Goldberg, 1985. *Black carbon in the environment*. Wiley and Sons.

Guggenberger, G., Rodionov, A., Shibistova, O., Grabe, M., Kasansky, O. a., Fuchs, H., Mikheyeva, N., Zrazhevskaya, G., and Flessa, H., 2008. Storage and mobility of black carbon in permafrost soils of the forest tundra ecotone in Northern Siberia. Global Change Biology 14:1367–1381.

Hamer, U., Marschner, B., Brodowski, S., and Amelung, W., 2004. Interactive priming of black carbon and glucose mineralisation. Organic Geochemistry 35:823–830.

Hammes, K., Smernik, R. J., Skjemstad, J. O., Herzog, A., Vogt, U. F., and Schmidt, M. W., 2006. Synthesis and characterisation of laboratory-charred grass straw (Oryza sativa) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. Organic Geochemistry 37:1629–1633.

Hammes, K., Torn, M., Lapenas, A., and Schmidt, M., 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeosciences 5:1339–1350.

Hilber, I., Blum, F., Leifeld, J., Schmidt, H.-P., and Bucheli, T. D., 2012. Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application. Journal of agricultural and food chemistry 60:3042–50.

Hilscher, A., Heister, K., Siewert, C., and Knicker, H., 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemistry 40:332–342.

Hilscher, A. and Knicker, H., 2011. Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology and Biochemistry 43:261–270.

Hockaday, W., Grannas, A., Kim, S., and Hatcher, P., 2006. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. Organic Geochemistry 37:501–510.

Intergovernmental Panel on Climate Change, 2013. Fifth assessment report. Technical report.

Janssens, I., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E.-D., Tang, J., and Law, B., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3:315–322.

Jeffery, S., Verheijen, F., van der Velde, M., and a.C. Bastos, 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment 144:175–187.

Jenkinson, D. S. and Rayner, J. H., 1977. The turnover of soil organic matter in some of the Rothamsted Classical Experiments. Soil Science 123:298–305.

Jones, D., Murphy, D., Khalid, M., Ahmad, W., Edwards-Jones, G., and DeLuca, T., 2011. Short-term biochar-induced increase in soil CO2 release is both biotically and abiotically mediated. Soil Biology and Biochemistry 43:1723–1731.

Jones, D., Rousk, J., Edwards-Jones, G., DeLuca, T., and Murphy, D., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biology and Biochemistry 45:113–124.

Keeling, C. D., 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta 13:322–324.

Keith, A., Singh, B., and Singh, B., 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. Environmental Science & Technology 45:9611–8.

Khirkham, D. and Bartholomew, W. V., 1954. Equations for Following Nutrient Transformations in Soil. Soil science society proceedings 1:33–34.

Kimetu, J. M. and Lehmann, J., 2010. Stability and stabilisation of biochar and green manure in soil with different organic carbon contents. Australian Journal of Soil Research 48:577.

Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85:91–118.

Knicker, H., 2011. Soil organic N - An under-rated player for C sequestration in soils? Soil Biology and Biochemistry 43:1118–1129.

Knicker, H., González-Vila, F. J., and González-Vázquez, R., 2013. Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biology and Biochemistry 56:31–39.

Kolb, S. E., Fermanich, K. J., and Dornbush, M. E., 2009. Effect of Charcoal Quantity on Microbial Biomass and Activity in Temperate Soils. Soil Science Society of America Journal 73:1173.

Kuhlbusch, T. A. J. and Crutzen, P. J., 1995. Toward a global estimate of black carbon in residues of vegetation fires representing a sink of atmospheric CO2 and a source of 02. Global Biogeochemical Cycles 9:491–501.

Kuhlbusch, T. A. J., Crutzen, P. J., and Planck, M., 1995. Toward a global estimate of black carbon in residues of vegetation fires representing a sink of atmospheric CO2 and a source of 02. Global Biogeochemical Cycles 9:491–501.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., and Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biology and Biochemistry 41:210–219.

Lehmann, J., 2007. Bio-energy in the black. Frontiers in Ecology and the Environment 5:381–387.

Leifeld, J., Fenner, S., and Müller, M., 2007. Mobility of black carbon in drained peatland soils. Biogeosciences 4:425–432.

Liang, B., Lehmann, J., Sohi, S., Thies, J., ONeill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E., and Luizão, F., 2010. Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry 41:206–213.

Lindbeck, M. R. and Young, J. L., 1965. Polarografy of intermediates in the fixation of Nitrogen by p-quinone-acqueous ammonia systems. Analytical Chemical Acta 32:73–80.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., and Brookes, P., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology and Biochemistry 43:2304–2314.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P. G., Kolari, P., Kowalski, A. S., Lankreijer, H., Law, B. E., Lindroth, A., Loustau, D., Manca, G., Moncrieff, J. B., Rayment, M., Tedeschi, V., Valentini, R., and Grace, J., 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447:848–50.

Major, J., Lehmann, J., Rondon, M., and Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16:1366–1379.

Masiello, C., 2004. New directions in black carbon organic geochemistry. Marine Chemistry 92:201–213.

Miller, J. D., Knapp, E. E., Key, C. H., Skinner, C. N., Isbell, C. J., Creasy, R. M., and Sherlock, J. W., 2009. Calibration and validation of the relative differenced Normalized Burn Ratio (RdNBR) to three measures of fire severity in the Sierra Nevada and Klamath Mountains, California, USA. Remote Sensing of Environment 113:645–656.

Moritz, M., Parisien, M., Batllori, E., Krawchuk, M. A., Van Dorn, J., Ganz, D. J., and Hayhoe, K., 2012. Climate change and disruptions to global fire activity. Ecosphere 3:1–22.

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M. C., and Marinari, S., 2012. Soil enzymology: classical and molecular approaches. Biology and Fertility of Soils 48:743–762.

Nelissen, V., Rütting, T., Huygens, D., Staelens, J., Ruysschaert, G., and Boeckx, P., 2012. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biology and Biochemistry 55:20–27.

Nguyen, B. T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S. J., and Engelhard, M. H., 2008. Long-term black carbon dynamics in cultivated soil. Biogeochemistry 89:295–308.

Nömmik, H. and Vahtras, K., 1982. Retention and fixation of NH4+ and NH3. Agronomy monographs 22:152–169.

Novak, J., Busscher, W., Watts, D., Laird, D., Ahmedna, M., and Niandou, M., 2010. Short-term CO2 mineralization after additions of biochar and switchgrass to a Typic Kandiudult. Geoderma 154:281–288.

Olson, J. S., 1963. Energy Storage and the Balance of Producers and Decomposers. Ecology 44:322–331.

Paterson, E., Midwood, A. J., and Millard, P., 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. The New phytologist 184:19–33.

Pessenda, L. C. R., Gouveia, S. E. M., and Aravena, R., 2001. Radiocarbon dating of total soil organic matter and humin fraction and its comparison with 14C ages of fossil charcoal. RADIOCARBON 43:595–601.

Pietikäinen, J., Kiikkilä, O., and Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89:231–242.

Reich, P. B. and Hobbie, S. E., 2012. Decade-long soil nitrogen constraint on the CO2 fertilization of plant biomass. Nature Climate Change 3:278–282.

Rumpel, C., Chaplot, V., Planchon, O., Bernadou, J., Valentin, C., and Mariotti, A., 2006. Preferential erosion of black carbon on steep slopes with slash and burn agriculture. Catena 65:30–40.

Santos, F., Torn, M., and Bird, J. A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology and Biochemistry 51:115–124.

Schmidt, M. and Noack, A., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14:777–793.

Schmidt, M., Torn, M., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D., Nannipieri, P., Rasse, D., Weiner, S., and Trumbore, S., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478:49–56.

Schneider, M. K., Lüscher, A., Richter, M., Aeschlimann, U., Hartwig, U. a., Blum, H., Frossard, E., and Nösberger, J., 2004. Ten years of free-air CO 2 enrichment altered the mobilization of N from soil in Lolium perenne L. swards. Global Change Biology 10:1377–1388.

Schneider, M. P., 2011. *Assessment of a Molecular Marker Method to Determine the Pyrogenic Carbon Component in Charcoals and Soils*. Ph.D. thesis, University of Zurich.

Schneider, M. P., Lehmann, J., and Schmidt, M. W., 2011. Charcoal quality does not change over a century in a tropical agro-ecosystem. Soil Biology and Biochemistry 43:1992–1994.

Seiler, W. and Crutzen, P. J., 1980. Estimates and gross and net fluxes of carbon between the biosphere and the atmosphere from biomass burning. Climate Change 2:207–247.

Singh, B. P., Cowie, A. L., and Smernik, R. J., 2012a. Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. Environmental Science & Technology 46:11770–8.

Singh, N., 2013. *Dynamics of Pyrogenic Organic Matter: Physical, Chemical and Biological Transformation and Stabilization Processes in the Soil*. Ph.D. thesis, University of Zurich.

Singh, N., Abiven, S., Torn, M., and Schmidt, M., 2012b. Fire-derived organic carbon turnover in soils on a centennial scale. Biogeoscience 9:1–11.

Sinsabaugh, R., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42:391–404.

Skjemstad, J., Clarke, P., T., J.A., O., and J.M., McClure, S., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Research1 34:251–271.

Spokas, K. a., Koskinen, W. C., Baker, J. M., and Reicosky, D. C., 2009. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77:574–81.

Ste-Marie, C. and Pare, D., 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology and Biochemistry 31:1579–1589.

Steinbeiss, S., Gleixner, G., and Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biology and Biochemistry 41:1301–1310.

Steiner, C., Glaser, B., Geraldes Teixeira, W., Lehmann, J., Blum, W. E., and Zech, W., 2008. Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. Journal of Plant Nutrition and Soil Science 171:893–899.

Thornton, P. E., Doney, S. C., Lindsay, K., Moore, J. K., Mahowald, N., Randerson, J. T., Fung, I., Lamarque, J.-F., Feddema, J. J., and Lee, Y.-H., 2009. Carbon-nitrogen interactions regulate climate-carbon cycle feedbacks: results from an atmosphere-ocean general circulation model. Biogeosciences 6:2099–2120.

Vasilyeva, N. a., Abiven, S., Milanovskiy, E. Y., Hilf, M., Rizhkov, O. V., and Schmidt, M. W., 2011. Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. Soil Biology and Biochemistry 43:1985–1988.

Wang, J., Pan, X., Liu, Y., Zhang, X., and Xiong, Z., 2012. Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. Plant and Soil 360:287–298.

Wardle, D. A., Nilsson, M.-C., and Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320:629.

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., and Joseph, S., 2010. Sustainable biochar to mitigate global climate change. Nature communications 1:56.

Woolf, D. and Lehmann, J., 2012. Modelling the long-term response to positive and negative priming of soil organic carbon by black carbon. Biogeochemistry 111:83–95.

Zavalloni, C., Alberti, G., Biasiol, S., Vedove, G. D., Fornasier, F., Liu, J., and Peressotti, A., 2011. Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. Applied Soil Ecology 50:45–51.

Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J., and Zhang, X., 2011. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant and Soil 351:263–275.

Zimmerman, A. R. and Gao, B., 2013. The stability of biochar in the environment. In N. Ladygina and F. Rineau, editors, *Biochar and soil biota*, chapter 1, pages 1–40. CRC press, Boca Raton.

Zimmerman, A. R., Gao, B., and Ahn, M.-Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biology and Biochemistry 43:1169–1179.

Zimmermann, M., Bird, M. I., Wurster, C., Saiz, G., Goodrick, I., Barta, J., Capek, P., Santruckova, H., and Smernik, R., 2012. Rapid degradation of pyrogenic carbon. Global Change Biology 18:3306–3316.

Ziolkowski, L. A. and Druffel, E. R. M., 2010. Aged black carbon identified in marine dissolved organic carbon. Geophysical Research Letters 37:4–7.

Chapter B

Manuscripts

1 Manuscript I

Carbon losses from pyrolysed and original wood in a forest soil under natural and increased N deposition

Bernardo Maestrini, Samuel Abiven, Nimisha Singh, Jeffrey Bird, Margaret S. Torn, Michael W.I. Schmidt

Manuscript currently under discussion in *Biogeoscience*.

Candidate contribution: The candidate performed measurements, data analysis, writing of the manuscript and field maintanance. The experimental set-up was conceived and realized by Dr. Samuel Abiven.

Carbon losses from pyrolysed and original wood in a forest soil under natural and increased N deposition.

Bernardo Maestrini[1], Samuel Abiven[1*], Nimisha Singh[1],
Jeffrey Bird[2], Margaret S. Torn[3], Michael W.I. Schmidt[1]

1: Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057, Zurich, Switzerland;
2: School of Earth and Environmental Sciences, Queens College, City University of New York, 11367, Flushing New York, USA;
3: Lawrence Berkeley National Laboratory, Earth Science Division, 94720, Berkeley, USA.
* Corresponding author: samuel.abiven@geo.uzh.ch, tel. +41-(0)44 635 51 83

Keywords: Pyrogenic Organic Matter, Dissolved Organic Carbon, Soil respiration, Mineral Nitrogen addition.

Abstract

Pyrogenic organic matter (PyOM) plays an important role as a stable carbon (C) sink in the terrestrial ecosystems. However, uncertainties remain about *in situ* turnover rates of PyOM in soil, the main processes leading to PyOM C and nitrogen (N) losses from the soil, and the role of N availability in PyOM cycling in soils.

We measured PyOM and native soil organic carbon losses from the soil as carbon dioxide and dissolved organic carbon (DOC) using additions of highly ^{13}C-labelled PyOM (2.03 atom %) and its precursor pinewood during one year in a temperate forest soil. The field experiment was carried out under ambient and increased mineral N deposition (+ 60 Kg N ha^{-1} $year^{-1}$). The results showed that after one year: 1) 0.5% of PyOM-C and 22% of wood-C were mineralized as CO_2, leading to an estimate of minimum turnover time of 191 and 4 years respectively, 2) the quantity of PyOM and wood lost as dissolved organic carbon was negligible (0.0004±0.0003 % and 0.022±0.007 respectively); and 3) N additions decreased cumulative PyOM mineralization by 43%, but did not affect cumulative wood mineralization and did not affect the loss of DOC from PyOM or wood. We conclude that mineralization to CO_2 was the main process leading to PyOM losses during the first year of decomposition in a forest soil, and that N addition can decrease PyOM C cycling while leaving unaltered wood C cycling.

1. Introduction

Pyrogenic organic matter (PyOM) is the product of incomplete combustion of biomass (Goldberg, 1985) and is an important soil C pool because it can represent up to 45% of soil organic carbon (Schmidt et al. 1999). Moreover, PyOM input from biomass burning is likely to increase in high-to-mid–latitude-regions in the future (Westerling et al. 2006; Moritz et al. 2012), because climatic conditions favouring fire will probably become more frequent. Due to its aromatic structure, PyOM has been hypothesized to be particularly resistant to microbial decomposition (Schmidt and Noack, 2000) and have a centennial mean residence time (Schmidt et al. 2011; Singh et al. 2012).

Several recent publications have investigated PyOM decomposition rate under controlled laboratory conditions (Hilscher et al. 2009; Kuzyakov et al. 2009; Abiven and Andreoli, 2010; Santos et al. 2012). However, only a few field experiments have been conducted, mainly because the decomposition rate of PyOM is difficult to detect without dedicated techniques such as isotopic tracers and/or biomarkers. Most field studies have been observational, comparing the PyOM content of archived soils or chronosequences. Hammes et al. (2008) compared the change in PyOM in a Chernozem sampled twice at 100 years

interval and found a loss of 25% using the benzene polycarboxylic acid (BPCA) technique. Using a chronosequence of soils that underwent slash and burn at different times over the last 100 years, Nguyen et al. (2008) found a loss of 30% soil PyOM over this period; the authors used the NMR technique for analysing pre-oxidized handpicked samples. For the same samples using the BPCA technique, Schneider et al. (2011) observed no change in the PyOM content of soils that received PyOM deposition for 2 to 100 years. This approach of using archived or chronosequence samples is limited by the lack of information regarding the amount of initial input of C to the soil and the changes in edaphic conditions during the decomposition. An alternative method that overcomes the need to analytically isolate PyOM is to use stable isotopes, which allows the fate of added PyOM to be traced over time. Major et al. (2010) used this approach in a field trial in a tropical ecosystem. They added PyOM produced from mango tree (C_3 plant) to a grassland soil in Colombia (soil organic matter derived from C_4 plants), and by measuring soil respiration and ^{13}C-CO_2 found that 2.2% of the added PyOM-C was lost as CO_2 over two years. However, due to the slow rate of decomposition of PyOM, the difference in ^{13}C due to the plant photosynthetic pathways was not enough to observe a significant change in the ^{13}C content of CO_2 efflux, even though they observed an increase in soil respiration (SR). The study by Major et al. (2010), who found that losses as DOC were lower than 1% of the initially added PyOM-C, is the only study to our knowledge, that has assessed the mineralization and leaching losses of PyOM.

Singh et al. (2012) reviewed PyOM mean residence time by compiling a database with results from studies using different experimental designs. One clear message was that PyOM mean residence time was longer in field studies than in incubation studies, but the reason for that could not be reduced to one single factor. First, the constant temperature and moisture conditions in the laboratory may increase the decomposition of PyOM-C together with mixing soil and substrate at the beginning of the experiment. Second the short duration of incubation studies might overestimate turnover time of PyOM, because short-term experiments capture only the initial higher decomposition rate of PyOM (Hamer et al. 2004, Kuzyakov et al. 2009). Third, specific stabilisation processes, might need particular climatic conditions, for example freeze-thawing or disaggregation - aggregation, which usually occur under field conditions. The lack of short-term field manipulation experiments, does not allow disentangling this conundrum.

Losses of PyOM from soil have been shown to also occur as dissolved organic carbon (DOC), however there are contrasting evidences on the importance of this pathway for PyOM-C losses. Consistent losses of PyOM as DOC were observed by Leifeld et al. (2007) who reported that 21-69% of PyOM moved below the ploughing depth (30 cm) over at least 50 years, in a former agricultural soil where combustion residues were disposed. Substantial quantities of PyOM have been detected in riverine (Kim et al. 2004) and marine (Thorsten Dittmar, 2008; Ziolkowski and Druffel, 2010) DOC. Abiven et al. (2011) found that the soluble fraction in PyOM was 1 g C kg^{-1} PyOM-C, while PyOM aged in the field (10 years) contained a much higher fraction of the soluble PyOM, with a value of 41 g C kg^{-1} PyOM-C. Major et al. (2010), reported PyOM losses as DOC of 0.2 g C kg^{-1} PyOM-C over a two year

period. Moreover, the lack of data on losses of PyOM-C as CO_2 and DOC derived from same experiment leaves unresolved the question on the relative importance of the two processes (Bird et al. 1999; Hammes et al. 2008).

Also soil erosion can also cause losses of PyOM from a given location or watershed, mainly due to its low bulk density. For example Rumpel et al. (2006) found that PyOM-C can represent up to 30% of eroded carbon in a tropical watershed. However, erosion losses were not determined in the present study.

It has been also observed that PyOM may change the turnover rates of the native soil organic matter (SOM) already present in the soil. PyOM additions to soil has been found to increase (Wardle et al. 2008; Major et al. 2010; Luo et al. 2011), decrease (Liang et al. 2006; Cross and Sohi, 2011) or have no effect (Kuzyakov et al. 2009; Cross and Sohi, 2011; Santos et al. 2012) on the decomposition rate of the native soil organic matter in soils. Keith et al. (2011) found that PyOM increased the decomposition of native soil organic matter and decreased the decomposition of fresh organic matter added along with the PyOM. They suggested that small portions of labile PyOM may trigger the decomposition of the native soil organic matter, while fresh organic matter is incorporated in the microaggregates whose formation is promoted by PyOM (Liang et al. 2010). This change in the SOM decomposition rate, also called priming effect, is an important feedback mechanisms than can modify the carbon stocks in soils. Positive priming effect (an increase in SOM decomposition) has been related to the PyOM particles serving as a surface for microbial growth (Wardle et al. 2008), to the increased root inputs from plants following PyOM inputs (Major et al. 2010), or to the liming effect of PyOM addition (Luo et al. 2011). A negative priming effect has been explained by the adsorption of organic matter on PyOM surface in a way that protects it from mineralization (Liang et al. 2006; Cross and Sohi, 2011).

In addition to its chemical structure, PyOM decomposition may be affected by other drivers of the ecosystem, such as inorganic nitrogen (N) availability. Increased N deposition has been observed to have contradicting effects on soil organic matter dynamics, increasing (Burton et al. 2004) or decreasing (Pregitzer et al. 2007) decomposition rates. Where observed, reduced mineralization has been explained by a decrease in the activity of ligninolitic enzymes (Carreiro et al. 2000; Sinsabaugh, 2010) which could particularly affect PyOM given its aromatic structure. Alternatively a shift in microbial decomposers community toward more efficient C-user microbes has been suggested (Janssens et al. 2010) to be responsible for decreased soil respiration.

We added ^{13}C-labelled PyOM and ^{13}C-labelled wood parent material, *Pinus Ponderosa*, to the soil of a forest clearing, with two levels of N addition (0 and +60 kg N ha^{-1} $year^{-1}$) to trace losses of C as CO_2 and as DOC, and to directly estimate priming effect. The focus was on the following research questions: (1) What is the CO_2 mineralization rate of PyOM compared to that of the un-pyrolized wood substrate? (2) How much PyOM is lost as DOC? (3) Does PyOM induce a priming effect on native soil organic matter? (4) What is the effect of N deposition on soil C and PyOM-C fluxes from the soil?

2. Materials and methods

2.1 Field Study

The field experiment was located in a temperate forest adjacent to a CarboEurope forest tower on the Laegern mountain (CH-LAE, 47°28'42.0"N; 8°21'51.8"E, altitude is 700 m a.s.l., mean annual soil temperature 10 ºC), 20 km from Zurich, Switzerland. The overstory vegetation consists mainly of beech (*Fagus sylvatica L.*), spruce (*Picea Abies L.*), ash (*Fraxinus excelsior L.*), fir (*Abies alba Mill.*), and small-leaved lime (*Tilia Cordata Mill.*), and understory of *Allium ursinum (L.)* (Ruehr et al. 2009). The soil is a Cambisol (sand: 45%, silt: 24%, clay: 31%), rich in organic C (34 g C kg^{-1}, Table 1).

The experimental design was a 3x2 factorial experiment, we tested three organic input treatments (control - corresponding to no input, wood, and PyOM) and two N addition levels (ambient and +60 kg N ha^{-1} $year^{-1}$). The experiment was set-up in three blocks each containing all the possible combinations of input treatment and N addition, i.e. the experiment was composed of three replicates. The experimental blocks were located in a forest gap of approximately 0.5 ha. We installed 18 mesocosms, each consisting of 20-cm long, 10-cm diameter polyethylene cylinders which were inserted in the soil down to a depth of ca. 15 cm with 5 cm above the soil surface. Each mesocosm had an open bottom and had two 0.7 mm-mesh covered "windows" (4–cm diameter) placed at distances of 7.5 cm and 12.5 cm distance from the bottom. These windows were added to the mesocosms to allow biological, chemical, and climatic equilibration with the external environment, without coarse roots entering. Mesocosms were installed in April 2009 and labelled organic input addition occurred in October 2009.

Any grass and roots growing inside the mesocosms were removed by clipping each time CO_2 was sampled (see the section on CO_2 efflux) to avoid including autotrophic respiration in the measurements.

Half of the mesocosms received N fertilization (60 kg N-NH_4NO_3 ha^{-1} yr^{-1}) with 5 kg ha^{-1} applied every month (10 kg ha^{-1} was applied exceptionally three times to catch up with application delay due to the accessibility of the research site). N was first added to soil in March 2010, 6 months after the application of organic inputs. In each block at a depth of 5 cm we placed a sensor measuring temperature and soil moisture (5TM soil moisture sensor, Decagon, USA). The data collected prior to March 2010 were thus not affected by the N fertilization.

2.2 Organic inputs

The pine wood and PyOM added to the soil mesocosms were isotopically enriched in ^{13}C (see Table 2). Two-years-old pine saplings (*Pinus Ponderosa*) were labelled with $^{13}C–CO_2$ under controlled greenhouse conditions. They were exposed to ten photoperiods of enriched

$^{13}C–CO_2$ (10 atom %) during the course of their growth (Bird and Torn, 2006). PyOM was produced by pyrolyzing the labelled wood at 450 °C for 5 h under N_2 atmosphere, as described in (Santos et al. 2012). The PyOM and wood were subsequently milled (< 2 mm) and their C, N, and ^{13}C content analysed (see Table 2). The equivalent of 397 g C m^{-2} of PyOM and 189 g C m^{-2} of wood were placed at a soil depth of 1 cm within mesocosms, and the soil was mixed. The soil in the control mesocosms was also mixed to 1 cm. The detailed chemical composition, structure, and isotopic content of the pine wood and PyOM used in this study are described in (Chatterjee et al. 2012). Chatterjee et al. (2012) report that the pyrolisis of wood lead to a depolymerisation of hemicelluloses and cellulose which were less present in the char than in the wood. Unfortunately their study did not allow concluding on the fate of ligneous components of wood, which may be partially or totally transformed into new aromatic compounds during the charring process.

2.3 Soil respiration

Soil respiration (SR) was measured 16 times during one year using a Li-Cor 8100 equipped with a chamber (10 cm diameter, 854.2cm^3 volume) on the following sampling dates: 6, 13, 20 November in 2009; 31 March, 8 and 29 April, 18 May, 4 and 22 June, 9 and 20 July, 4 and 26 August, 16 September, 4 October, 4 November in 2010. The hiatus between November and March 2009 is due to snow covering the soil.

Soil respiration was measured three times per mesocosm on each sampling date for 90 seconds. To allow the re-equilibration of the CO_2 concentration between the chamber and the atmosphere measurements were taken 90 seconds apart. At the same time, we also collected samples to measure the ^{13}C enrichment of soil respired CO_2. A Keeling plot approach (Keeling, 1958) was used to estimate the ^{13}C enrichment of the soil-respired CO_2 and to calculate the ^{13}C excess efflux from enriched PyOM and wood C. An 800 cm^3 chamber was placed on top of the mesocosms and CO_2 was sampled with a syringe three times at regular intervals over 9 minutes while the CO_2 increased. To produce each keeling plot we took three gas samples, collected after 0, 4.5, and 9 minutes, corresponding to a total average interval of 246 ppm between the gas sample taken at minute 0 and the one taken at minute 9. We sampled 17 ml of gas from the chamber, which was injected into a 12 ml vial (*Exetainer, Labco, United Kingdom*) using overpressure as suggested in Joos et al. (2008). Prior to sampling, the gas vials were flushed with N_2 three times, and stored under N_2. The ^{13}C enrichment of the CO_2 samples was measured using an isotope ratio mass spectrometer (IRMS) (*Delta-S, Finnigan MAT- Thermofscher scientific,Waltham, USA*). Keeling plots were accepted if r^2 of the regression line was higher than 0.9, if $r^2<0.9$ the data were discarded. A correction of $\delta^{13}C$ -4.4 ‰ was applied to the soil ^{13}C-CO_2 efflux for isotopic discrimination processes occurring during CO_2 diffusion from the soil as suggested by Mortazavi et al. (2004). Where data for soil respiration and ^{13}C-CO_2 were missing due to

technical failure they were replaced by the average of the same day of the corresponding treatment.

2.4 Dissolved organic carbon

Suction lysimeters (multilayer borosilicate 1 µm pore 10-cm diameter, *Ecotech, DH)* were placed at the bottom of the mesocosm (at a depth of 15 cm) and kept under constant vacuum at 650 to 750 mbars. Soil water was collected 9 times over the study period, on the following dates:

27 November, and 17 December in 2009, 14 January, 26 February, 13 May, 22 June, 24 August and 26 November in 2010.

Water samples were filtered using a 0.45 µm cellulose acetate membrane. $DO^{13}C$ measurement failed on two dates (27 May and 22 June) and since not enough material was left to repeat the measurement, these two sampling dates were discarded. The solution pH and electrical conductivity were measured (*Metrohm, 620 pH meter, Switzerland, and WTW tetracon, 325, Austria)* and samples after filtration stored at 4°C for maximum three weeks. Total organic carbon content was measured on a 20 ml subsample of the leachate. A second subsample of 60 ml was freeze-dried after removing potential carbonate by lowering pH to 2.8±0.1. Freeze-dried DOC (2–4 mg) samples were analyzed for total C using an elemental analyzer (*Shimatzu, Asi-v, Kyoto, Japan*) and ^{13}C enrichment was measured with an IRMS *(Delta-S, Finnigan MAT- Thermofscher scientific,Waltham, USA)*, coupled to an elemental analyzer (*EA 1100, Carlo Erba, Italy*). Where data for DOC and $DO^{13}C$ were missing due to technical failure they were replaced by the average of the same day of the corresponding treatment. If the fraction derived from the substrate (Equation 1 was below 0), then this was set to 0.

2.5 Calculations

The partitioning of the CO_2 and DOC fluxes between native SOM-derived and organic-input-derived (wood and PyOM) was calculated using Equation 1 from Balesdent and Mariotti, (1996):

$$f = 1 - (\delta^{13}C_{mix} - \delta^{13}C_{\text{organic input}}) / (\delta^{13}C_{control} - {}^{13}\delta C_{\text{organic input}}) \quad [1]$$

Where f = fraction of CO_2 flux derived from the organic input; $\delta^{13}C_{mix}$ = the isotopic signature of soil CO_2 or DOC in organic inputs treatments, $\delta^{13}C_{\text{organic input}}$ = the isotopic signature of the added organic inputs (PyOM and wood), and, $\delta^{13}C_{control}$ = the isotopic signature of soil CO_2 or DOC in the control treatment. The priming effect, i.e the change in native SOM mineralization due to the organic input was calculated for each sampling date as in Equation 2:

$$PE = (SR_{native} - SR_{control}) \quad [2]$$

Where $SR_{control}$ = soil respiration in the control treatment and SR_{native}=mineralization rate of the native soil organic matter in PyOM or wood treatment, calculated as: SR_{total}-(SR_{total}*f) where SR substrate is the total soil respiration in the substrate treatment.

To interpolate soil respiration between sampling dates, we modelled the CO_2 fluxes according to the method proposed by Fang and Moncrieff, (2001) (Equation 3). Kammer et al. (2011) successfully applied this model to the same site area as our study, and Ruehr et al. (2009) showed that the soil moisture affects soil respiration only when moisture is lower than 15 volume % . Soil moisture was always higher than this value during our study period, so soil moisture dropped out of our model and soil respiration was calculated as:

SRinterpolated = a*(T - Tmin)*b [3]

where *T* = the soil temperature (°C) measured at a depth of 5 cm (30 minutes measurement interval), *Tmin* is the temperature at which SR is supposed to be 0 (in this case fixed to -20°C) *a*, and *b* are model parameters calculated for each single mesocosm. The simulated soil respirations for different treatments are shown in Figure 1. PyOM mineralization measurements were linearly interpolated between sampling dates using Equation 4 assuming that the fraction derived from PyOM varied linearly between dates:

PyOM mineralization rate = $SR_{interpolated}$ * $f_{interpolated}$ [4]

where $f_{interpolated}$ values were calculated by interpolating between subsequent sampling date values obtained from Equation 1 as in Ngao et al. (2005) and Kammer and Hagedorn (2011). The modelled organic input mineralization (Figure 1) and the fraction derived from the labelled organic substrate in the period November 2009-March 2011 in the increased N deposition treatments was made equal to the mineralization occurring in the ambient N in the correspondent period to stress the effect of N addition.

We fitted the initial and the remaining quantity (Table 3) of PyOM at the end of the experiment, calculated by cumulating values from Equation 4, to a first-order decay model to estimate the Mean Residence Time (MRT) of PyOM and pine wood, as in Singh et al. (2012). Decomposition at time t in the first order decay model is expressed as follows:

$-dC/dt=k*C_t$ [5]

where C is the size of PyOM-C or the wood-C pool. From this formula it is possible to derive the pool size at time t using Equation 6, assuming that the inputs to the system were equal to 0 and that losses as DOC were negligible:

$C_t = C_{t=0}\, e^{-k\,t}$ [6]

Where k is the constant decay rate, and then calculate the mean residence time (MRT), for each mesocosm as:

MRT=1/k [7]

2.6 Statistical analyses

The effects of treatment, N addition, and time were tested on the following variables: soil respiration, the fraction derived from added substrate in soil respiration and DOC, DOC daily

production rate, soil water conductivity and soil water pH, using repeated measures ANOVA and individual ANOVA procedures on individual sampling dates.

Two different repeated measures ANOVA were performed: one over the time period November 2009-November 2010 to test the effect of different organic inputs and time, and their interactions, and another was performed over the period March 2010-November 2010 (during the N addition period, started in March 2010). Individual comparisons within the same organic treatment and the N-addition treatment were performed using Tukey's comparison to test differences among sampling dates.

T-tests were performed on individual sampling dates (ambient and increased N treatment pooled together) to test whether the fraction derived from CO_2 was significantly different from 0.

Repeated measures ANOVA was performed using *SPSS Statistics v.18* (IBM, New York, USA) and Tukey's comparison tests were performed using *R* (version 2.10), extended by the "agricolae" package.

3. Results

3.1 Soil respiration rates, organic input mineralization, and priming effect

The average soil respiration rate (SR) in the control treatment (no additions) during the year was 2.0±0.1 μmol CO_2 m^{-2} s^{-1}. Neither N nor organic input addition significantly affected the soil respiration rates whereas time did, with higher respiration rates measured in July and September (repeated measures ANOVA $p<0.001$, Figure 1).

The fraction of soil respiration derived from the substrate was significantly higher in the wood treatment than in PyOM for all sampling dates (Post-Hoc Tukey test $p<0.05$, Figure 3). Nitrogen addition did not have a significant effect on the fraction derived from the added substrate.

Under ambient N the fractions of soil respiration derived from PyOM on 6 November 2009 was significantly different from that measured on 18 May 2010. Under increased N, during the period March 2010-November 2010, we observed no differences in the PyOM derived fraction of soil respiration among sampling dates (Post-Hoc Tukey test. $p<0.05$, Figure 3 a).

For the wood treatment under ambient N, the wood derived fraction of the 6 and 20 of November 2009 was significantly higher than the one measured between June and November 2010. Under increased N, during the period March-November 2010, only the fraction derived from wood on the 31 March was significantly different from the following ones (Post-Hoc Tukey test. $p<0.05$, Figure 3 b).

The PyOM decomposition rate was on average 0.0022±0.0003 % and 0.0011±0.0002 % of PyOM-C day^{-1} added under ambient and increased N, respectively. Wood decomposition rate was 0.077±0.008 and 0.081±0.008 day^{-1} of % wood-C added under ambient and increased N, respectively.

N addition did not significantly affect the decomposition of the substrates. On individual sampling dates N addition significantly decreased PyOM decomposition only on the 20 July, while wood decomposition was not affected by N addition at any stage (Figure 4). No significant difference in mineralization rate was observed among sampling dates.
The mineralization of native soil organic matter, calculated as soil respiration minus organic substrate-derived CO_2 in the substrate-addition treatment or raw soil respiration in the control treatment, was not significantly affected by either the addition of the organic inputs or the addition of N, i.e. no priming was observed (Figure 6, repeated measures ANOVA, $p<0.05$). However, under ambient N the PyOM induced priming effect tends to be higher.
Using Equation 4 we estimated the quantity of PyOM-C and wood-C lost as CO_2 over one year. PyOM-C losses were 0.5 ± 0.1 % of initial PyOM-C and wood-C losses were 22 ± 3% of initial wood-C input. N addition significantly decreased the PyOM-C decomposition to 0.3±0.1%, (t-test, $p<0.05$), while wood decomposition was not affected by N addition (Figure 5, Table 3). The MRT calculated according to Equation [7] was 191 ± 24 and 430 ± 146 years for PyOM-C for ambient and increased N, respectively and 4±1 years for Wood-C for both ambient and increased N treatment.

3.2 Dissolved organic carbon

DOC production rate did not significantly differ with treatment and different levels of N added, and nor did its pH, conductivity, or volume (data not shown). The fraction of DOC derived from the substrate was significantly different between the wood and PyOM (repeated measures ANOVA, $p<0.05$), under increased and under ambient N.

Cumulative losses of PyOM-C were 0.0004±0.0003% of the initial input-C, while wood-C losses were 0.022±0.007 % of the wood-C, and under increased N addition losses were 0.0002±0.0001% for PyOM, and 0.03±0.01% for wood (Figure 7; Table 3). Nitrogen did not affect the cumulative losses of DOC neither in the PyOM and nor in the wood treatment.

4. Discussion

4.1 PyOM and wood mineralization

In our study, we estimated that PyOM in the field has a turnover time of 191 years, while wood had a turnover time of 4 years. PyOM turnover time in our field study was higher than the turnover time for wood derived PyOM found in incubation studies (Hamer et al. 2004; Hilscher et al. 2009, Singh et al. 2012). Using the same substrate in an incubation experiment, Santos et al. (2012) found a decomposition rate of 0.39% after 180 days compared to 0.08% in our study after 180 days. The increased turnover time for PyOM decomposition than observed in the laboratory incubation studies indicate that higher and more constant temperature and soil moisture conditions are likely to increase the decomposition rate of PyOM compared to our field studies, whose mean annual soil temperature was 10 °C and moisture was 30 volume %.

The PyOM mean residence time calculated here is closer to the values reported by Nguyen et al. (2008), who found a mean residence time of 264 years under tropical climate and from Hammes et al. (2008) who found a mean residence time of 347 years in a boreal steppe, indicating that irrespective of the climate, the quantification method and the length of the experiment PyOM has a mean residence time ranging in the centennials, when measured in field conditions. On the other hand in a two-years field experiment carried out in a plant-soil system in a tropical savanna, Major et al. (2010) found a turnover time of 89 years. However in their experiment the presence of rhizodeposition, due to the increase in biomass (observed in the first year) following PyOM addition, may have primed the PyOM decomposition. In fact easily decomposable compounds like glucose or rhizodeposition can increase the PyOM decomposition rate, since enzymes produced for their decomposition can contribute also to the decomposition of PyOM (Hamer et al. 2004; Kuzyakov et al. 2009). A similar effect was observed by Keith et al. (2011), who found an increase in PyOM decomposition as result of the addition of fresh organic matter to the soil.

The PyOM mineralization rate did not decrease significantly with time (Figure 4). Kuzyakov et al. (2009) found that the PyOM mineralization rate decreased over the first two to three months before levelling off. Likewise, in an incubation in quartz medium, the PyOM mineralization rate was twice as fast in the first month as in the second month (Hamer et al. 2004). In our experiment PyOM decomposition rate tended to decrease (not significantly) over the first three weeks, and increased in summer, suggesting that temperature might have influenced PyOM decomposition (Fang et al. 2011). However the setup of our study did not allow distinguishing between the effect of the season and of time.

The fraction of PyOM-derived C in the soil respiration did not vary along the time of decomposition (Figure 3 a). The fraction was higher for the first three sampling date, but was rapidly levelled off. This confirms findings from Smith et al. (2010) who observed that the PyOM fraction in soil respiration rapidly decreased over one week in an incubation experiment. Using the fraction derived from PyOM as an indicator for PyOM decay relative to native soil organic carbon, our study indicates that the stability of PyOM relative to native soil organic matter was constant over time, and was not affected by the higher temperature, not confirming the theory proposed by Hartley and Ineson (2008) on the higher temperature sensitivity of resistant organic matter. Cumulative wood decomposition after one year was equal to 22% of the initially added wood-C. On the same site Kammer and Hagedorn, (2011) found similar values for beech twigs, 22 – 26% mineralization over one year at the soil surface. Using the same substrate Santos et al. (2010) found wood decomposition of 22% in an andesitic soil and 30% in a granitic soil over 180 days, while in our study only 10% of the initially added wood-C was decomposed over the same period; these results confirm that similar to the PyOM, the decomposition of wood-C is slower in field conditions than in laboratory incubation experiments. The fraction of total soil respiration coming from wood decreased with time (Figure 3 b). This agrees with findings from Kammer et al. (2011).

4.2 Priming effect:

We did not observe a significant change in native soil organic matter mineralization due to the addition PyOM or wood despite large differences observed between the means (Figure 6). This is mainly due to the large variability in soil respiration measured in the field as well as the limited number of replicates used in our study. However, we observed a trend towards positive priming effect for both organic inputs under ambient N deposition, and no effect under increased N deposition.

Neither Abiven and Andreoli, (2010) who incubated PyOM and litter in the same soil, nor Santos et al. (2012) who incubated the same substrate in different soils observed a priming effect. In contrast, Wardle et al. (2008) reported increased losses of native C in a 10-years litterbag experiment mixing PyOM and humus and Major et al. (2010) found that PyOM addition increased native soil organic matter respiration. One important difference between these studies is the addition rate, Wardle et al. (2008) added PyOM in a 50:50 ratio to humus litter bags, while Major et al. (2010) had an addition rate 6 times higher than our study. Also Keith et al. (2011), in an incubation experiment, found a positive priming effect on native soil organic matter adding a higher quantity of char to soil, in fact they added 20 mg PyOM g^{-1} soil, a quantity ten times higher than ours, considering a soil depth of 15 cm.

The amount of new substrate added compared to the initial soil organic matter amount should explain partially the changes in microbial activity in the soil (Fontaine et al. 2003). So we propose that the addition rate of PyOM might play a role in the size of priming effect.

4.3 Dissolved organic carbon

In our study, the losses of PyOM and wood as DOC were three orders of magnitude lower than their respective losses as CO_2 after one year (0.0004% vs 0.5 % and 0.02% vs 22% of initially applied PyOM-C and wood-C, respectively; Figure 7). The losses of wood-C as DOC were 50 times higher than the DOC losses of PyOM. In a field study in a savanna Oxisol, Major et al. (2010) found that DOC losses were 0.003% of the initial PyOM-C at a depth of 15 cm, i.e. ten times higher than observed in the present study. This discrepancy can be partially explained by the different operational definition of DOC used in the two studies. In fact while Major et al. (2010) considered as DOC particles with a size <0.7 μm, we considered as DOC particles with a size <0.45 μm. Major et al. (2010) found that PyOM increased the soil water fluxes, but we observed no such increase in our study, maybe because their PyOM application rate was 6 times higher than ours. Also the rainfall regime of Savanna, characterised by high and concentrated annual rainfall might have contributed to increase the level of PyOM lost by leaching, compared to temperate forest where rainfall are low and more distributed during the year. Finally, Major et al. (2010) studied the movement of PyOM in an Oxisol, that is characterised by high water infiltration rates (Major et al. 2010; Soil Survey Stuff 1999), while the soil in our field site was rich in clay that may have prevented water from quickly percolating through the soil profile. The DOC movements are

probably comparatively restricted in our soil, and so are the PyOM DOC. This would indicate that soil properties determine the leaching of PyOM – DOC. A similar conclusion was drawn by Leifeld et al. (2007) who observed consistent vertical movements of PyOM along the profile of three grassland soils (21-69% of PyOM-C moved below 30 cm over 50 years). They suggested that such high vertical movement is due to the high hydraulic conductivity of the soil, which is characterised by a high porosity as it was formerly a peatland.
Our findings on limited losses of PyOM derived DOC confirmed previous studies showing that only a small portion of fresh PyOM was soluble in water (Abiven et al. 2011). However, the soluble fraction of PyOM may increase with the degree of surface oxidation and ageing of PyOM (Hockaday et al. 2006; Abiven et al. 2011). Alternatively, it is possible that part of the dissolved PyOM is absorbed into the first layers of the soil, characterised by high clay content, and released in the following years. Therefore due to the PyOM ageing and partial release of the PyOM adsorbed on clay minerals, it is reasonable to expect that larger proportion of PyOM will be released as DOC in the future.
In a nearby experiment, Kammer et al. (2011) observed wood losses as DOC equivalent to 1.5% of initially applied wood-C over one year, however in their experiment wood was applied on the soil surface and therefore directly exposed to the rainfall, while in our case wood was incorporated in the first cm of the soil. Moreover they observed that wood-derived DOC was strongly decreasing from the litter layer to 5 cm depth. Such a decrease of DOC fluxes along the profile indicates that the adsorption of DOC on mineral surfaces could be an important mechanism in preventing the wood-derived DOC from percolating down to 15 cm (Kaiser and Georg Guggenberger, 2000).

4.4 Effect of Nitrogen addition

The addition of N had a significant effect on substrates decomposition only on one sampling date (Figure 4). However N decreased the cumulative PyOM decomposition by 47% after one year (Figure 5; Table 3) but had no effect on cumulative wood decomposition. Santos et al. (2012) incubated the same PyOM in different soils, and found no effect of N addition on PyOM decomposition. These different results could be explained by the difference in the setup (3 times lower N addition level and in a single addition at the beginning of the incubation). Soil properties may also play a role, Santos et al. (2012) suggest that the added N may have been adsorbed in the interlayers of the vermiculite present in their soil preventing it from being available to microbes.
Carreiro et al. (2000) and Sinsabaugh, (2010) observed that N addition decreased phenol-oxidase and peroxidase activities. Such enzymes are involved in the cleavage of aromatic compounds, and thus could affect breakdown of PyOM. However this hypothesis was not confirmed by a decrease of wood decomposition that is also supposed to be affected by the activity of oxidative enzymes.
Our results agree with the N mining theory (Craine et al. 2007). N addition might depress the decomposition of added substrate by fulfilling the N demand of microbes *"mining"* the

recalcitrant fraction of the substrate to obtain available N. Confirming this hypotheses a substantial mineralization of PyOM-N by microbes was observed by Santos et al. (2012) and Hilscher and Knicker, (2011).
Alternatively N addition might have induced a shift in microbial community, by lowering the fungal:bacterial ratio (Frey et al. 2004). However very little is known on the microorganisms responsible for PyOM decomposition Lehmann et al. (2011).
Wood decomposition was not affected by N deposition confirming findings of Hagedorn et al. (2003) who measured beech twigs decomposition in a neighbouring site. However they found that N addition altered the decomposition pattern of wood by depressing the wood decomposition from six to twelve months after N addition (i.e. in the same time frame of N addition in our experiment), confirming findings of Berg and Matzner, (1997). Such different result may be explained by the shorter duration of N addition in our experiment, six months instead of one year, supporting findings of Knorr et al. (2005) who observed that the effect of N addition was extremely low when lasting for less than six months.

5. Conclusions

We added ^{13}C-labelled PyOM or pine wood to a temperate forest soil with and without added inorganic N. In the first year we observed that:

- PyOM C mineralized at a rate of 0.5 % of applied C per year.
- PyOM losses as dissolved organic carbon (DOC) were three orders of magnitude smaller than their losses as CO_2.
- N addition depressed the decomposition of PyOM by 43% but did not alter wood decomposition neither the losses by leaching from wood or PyOM.
- There was no significant increase in soil CO_2 respiration of native SOC after PyOM or wood was added to soil.

Therefore, we conclude that this pine-derived PyOM-C has a centennial scale mean residence time, that N can decrease PyOM mineralization and that mineralization to CO_2 is the main process leading to PyOM losses in the first after PyOM addition.

References

Abiven, S., Andreoli, R., 2010. Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. Biology and Fertility of Soils 47, 111–114.

Abiven, S., Hengartner, P., Schneider, M., Singh, N., Schmidt, M.W.I., 2011. Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. Soil Biology and Biochemistry 43, 1615–1617.

Balesdent, J., Mariotti, A., 1996. Mass spectrometry of soils, in: Boutton T.W., Yamasaki, S.I. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York.

Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environmental reviews 5, 1–25.

Bird, J.A., Torn, M.S., 2006. Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361–382.

Bird, M.I., Moyo, C., Veenendaal, E.M., Lloyd, J., Frost, P., 1999. Stability of elemental carbon in a savanna soil. Global Biogeochemical Cycles 13, 923–932.

Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P., Zak, D.R., 2004. Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. Global Change Biology 10, 1080–1091.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhust, D.F., 2000. Microbial enzyme shift explain litter decay responses to simulated Nitrogen deposition. Ecology 81, 2359–2365.

Chatterjee, S., Santos, F., Abiven, S., Itin, B., Stark, R.E., Bird, J.A., 2012. Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry. Organic Geochemistry 51, 35–44.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–13.

Cross, A., Sohi, Saran P., 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biology and Biochemistry 43, 2127–2134.

Dittmar, T., 2008. The molecular level determination of black carbon in marine dissolved organic matter. Organic Geochemistry 39, 396–407.

Fang, C., Moncrieff, J.B., 2001. The dependence of soil CO_2 efflux on temperature. Soil Biology and Biochemistry 33, 155–165.

Fang, Y.Y., Singh, B., Singh, B.P., 2011. Stability of biochar in contrasting soils of Australia, in: APBC Kyoto 2011.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry 35, 837–843.

Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196, 159–171.

Goldberg, 1985. Black carbon in the environment. Wiley and Sons.

Hagedorn, F., Spinnler, D., Siegwolf, R., 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology and Biochemistry 35, 1683–1692.

Hamer, U., Marschner, B., Brodowski, S., Amelung, W., 2004. Interactive priming of black carbon and glucose mineralisation. Organic Geochemistry 35, 823–830.

Hammes, K., Torn, M.S., Lapenas, A.G., Schmidt, M.W.I., 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeosciences 5, 1339–1350.

Hilscher, A., Heister, K., Siewert, C., Knicker, H., 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemistry 40, 332–342.

Hilscher, A., Knicker, H., 2011. Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. Organic Geochemistry 42, 42–54.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2006. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. Organic Geochemistry 37, 501–510.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3, 315–322.

Joos, O., Saurer, M., Heim, A., Hagedorn, F., Schmidt, M.W.I., Siegwolf, R.T.W., 2008. Can we use the CO_2 concentrations determined by continuous-flow isotope ratio mass spectrometry from small samples for the Keeling plot approach? Rapid Communications in Mass Spectrometry 22, 4029–4034.

Kaiser, K., Guggenberger, Georg, 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry 31, 711–725.

Kammer, A., Hagedorn, F., 2011. Mineralisation, leaching and stabilisation of ^{13}C-labelled leaf and twig litter in a beech forest soil. Biogeosciences 8, 1043–1076.

Kammer, A., Schmidt, M.W.I., Hagedorn, F., 2011. Decomposition pathways of ^{13}C-depleted leaf litter in forest soils of the Swiss Jura. Biogeochemistry 108, 395–411.

Keeling, C.D., 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta.

Keith, A., Singh, B., Singh, B.P., 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. Environmental science & technology 45, 9611–8.

Kim, S., Kaplan, L.A., Benner, R., Hatcher, P.G., 2004. Hydrogen-deficient molecules in natural riverine water samples—evidence for the existence of black carbon in DOM. Marine Chemistry 92, 225–234.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 41, 210–219.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812–1836.

Leifeld, J., Fenner, S., Müller, M., 2007. Mobility of black carbon in drained peatland soils. Biogeosciences 4, 425–432.

Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry 41, 206–213.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black Carbon Increases Cation Exchange Capacity in Soils. Soil Science Society of America Journal 70, 1719.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology and Biochemistry 43, 2304–2314.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16, 1366–1379.

Moritz, M., Parisien, M., Batllori, E., Krawchuk, M.A., Van Dorn, J., Ganz, D.J., Hayhoe, K., 2012. Climate change and disruptions to global fire activity. Ecosphere 3, 1–22.

Mortazavi, B., Prater, J.L., Chanton, J.P., 2004. A field-based method for simultaneous measurements of the $\delta^{18}O$ and $\delta^{13}C$ of soil CO_2 efflux. Biogeosciences 1–9.

Ngao, J., Epron, B., Brechet, C., Granier, A., 2005. Estimating the contribution of leaf litter decomposition to soil CO_2 efflux in a beech forest using ^{13}C-depleted litter. Global Change Biology 1768–1776.

Nguyen, B.T., Lehmann, Johannes, Kinyangi, James, Smernik, R., Riha, S.J., Engelhard, M.H., 2008. Long-term black carbon dynamics in cultivated soil. Biogeochemistry 89, 295–308.

Pregitzer, K.S., Burton, A.J., Zak, D.R., Talhelm, A.F., 2007. Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. Global Change Biology 143–153.

Ruehr, N.K., Knohl, A., Buchmann, N., 2009. Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry 98, 153–170.

Rumpel, C., Chaplot, V., Planchon, O., Bernadou, J., Valentin, C., Mariotti, A., 2006. Preferential erosion of black carbon on steep slopes with slash and burn agriculture. Catena 65, 30–40.

Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology and Biochemistry 51, 115–124.

Schmidt, M.W.I., Noack, A.., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14, 777–793.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Ko, I., 1999. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50, 351–365.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D., Nannipieri, P., Rasse, D.P., Weiner,

S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Singh, N., Abiven, S., Torn, M.S., Schmidt, M.W.., 2012. Fire-derived organic carbon turnover in soils on a centennial scale. Biogeoscience 9, 1–11.

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology and Biochemistry 42, 2345–2347.

Soil Survey Stuff, 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. Washington DC.

Wardle, D.A., Nilsson, M.-C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320, 629.

Westerling, A.L., Hidalgo, H.G., Cayan, D.R., Swetnam, T.W., 2006. Warming and earlier spring increase western U.S. forest wildfire activity. Science (New York, N.Y.) 313, 940–3.

Ziolkowski, L.A., Druffel, E.R.M., 2010. Aged black carbon identified in marine dissolved organic carbon. Geophysical Research Letters 37, 4–7.

Figure Captions

Figure 1: Measured (full symbols) versus modelled (continuous line) soil respiration rate (in μmol CO_2 m^{-2} s^{-1}), for PyOM under ambient N (a), PyOM under increased N (b), wood under ambient N (c) and wood under increased N (d). Error bars represent the standard error of the mean (n=3) of the measured soil respiration.

Figure 2: Soil respiration rate (in μmol CO_2 m^{-2} s^{-1}) for control (a), PyOM (b) and wood (c). Error bars represent the standard error of the mean (n=3)

Figure 3: Fraction of soil respiration derived from PyOM (a) and from wood (b). The sampling dates within the same treatment with different letters (on the top) are significantly different ($p<0.05$ TukeyHSD test). The first line of letters refers to the ambient N treatment and the second to the increased N treatment. Error bars represent the standard error of the mean (n=3). Asteriscs (*) indicate when the fraction is significantly different from 0 (ambient

and increased N treatment were pooled together, n=6, $p<0.05$).

Figure 4: Mineralization rate (in μmol CO_2 m^{-2} s^{-1}) of wood (a) and PyOM (b). Empty symbols represent the treatment under ambient N and full symbols under increased N. Asteriscs (*) indicate the dates when the addition of N significantly affected substrate decomposition (paired t-test,$p<0.05$). Error bars represent the standard error of the mean (n=3).

Figure 5: Cumulative losses of PyOM-C (a) and wood-C (b) as CO_2 in % of the initial C input derived from equation 4. The black line represents losses under ambient N deposition and the grey line losses under increased N. Continuous line represent the mean and the dashed line the standard error of the mean (n=3).

Figure 6: Priming effect induced by PyOM (a) and wood (b) in μmol CO_2 m^{-2} s^{-1}, derived from equation 2. Full symbols represent the treatment under ambient N and the empty symbols represent the treatment under increased N. Error bars represent the standard error of the mean (n=3).

Figure 7: Cumulated losses as DOC for PyOM (a) and Wood (b) expressed as % of initially added organic input-C. Empty symbols represent the ambient N treatment and full symbols represent the increased N treatment. Error bars represent the standard error of the mean (n=3).

Table 1: Chemical and physical properties of the soil (0-15 cm depth) at the field site. The values presented are means of 3 replicates ± standard errrors.

Texture			**Bulk density**			**pH**	**CEC**	**Elemental analysis**		
%			g cm^{-3}				mmol kg^{-1}	g kg^{-1} soil		
Sand	Silt	Clay	0-5 cm	5-10 cm	10-15 cm			C	H	N
45.5± 3.5	24.2± 4.4	31.5± 2.4	1.20± 0.14	1.21± 0.32	1.60± 0.32	5.9 ± 0.5	74.3± 14.9	33.7± 4.8	8.9± 0.7	2.4± 0.2

Table 2: Characteristics of PyOM and pine wood added to the soil.

	C	**N**	**H**	**C/N**	**^{13}C**	**Application rate**
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(mass ratio)	(atom%)	(g C m^{-2})
PyOM	799	7.1	34.3	110	2.03	397
Wood	499	4.3	66.2	115	2.05	189

Table 3: Average total C losses over the study period ± standard error (n=3). The losses as DOC and DOC from the added inputs correspond to the cumulated losses over a year. The losses as CO_2 and DOC from the added input are calculated using Equation 3. Letters indicate where treatments are significantly different (pair wise paired t-test, $p<0.05$).

	Total DOC	**Cumulated losses from the added input as DOC**	**Total losses as CO_2**	**Cumulated losses over one year from the added input as CO_2**
	(g C m^{-2})	(% initial input-C)	(g C-CO_2 m^{-2})	(% initial input)
Ambient N				
Control	2.1±0.7a		503 ± 100 a	
PyOM	1.8±0.3a	0.0004±0.0003 a	653 ± 21 a	0.5 ± 0.1 a
Wood	1.7±0.3a	0.022±0.007 b	726 ± 189 a	22 ± 3 b
Increased N				
Control	1.5±0.2a		638 ± 33 a	
PyOM	1.6±0.6a	0.0002±0.0001 a	650 ± 119 a	0.3 ±0.1 c
Wood	2.4±2.2a	0.03±0.01 b	715 ± 125 a	22 ± 3 b

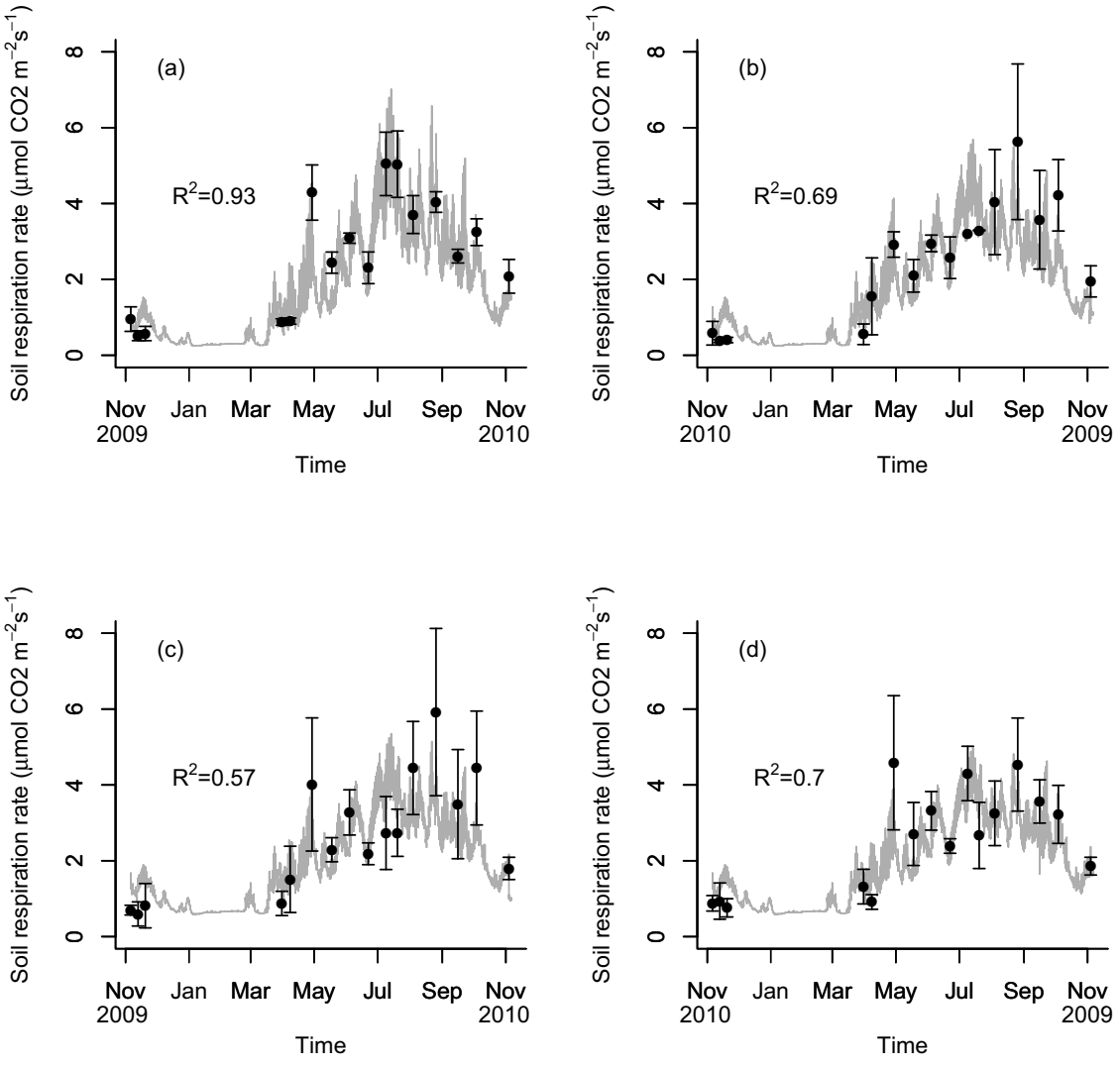

(a)
R2=0.93
(b)
R2=0.69
(c)
R2=0.57
(d)
R2=0.7
Soil respiration rate (µmol CO2 m−2s−1)
0
2
4
6
8
Nov 2009
Jan
Mar
May
Jul
Sep
Nov 2010
Nov 2010
Nov 2009
Time

Figure 1

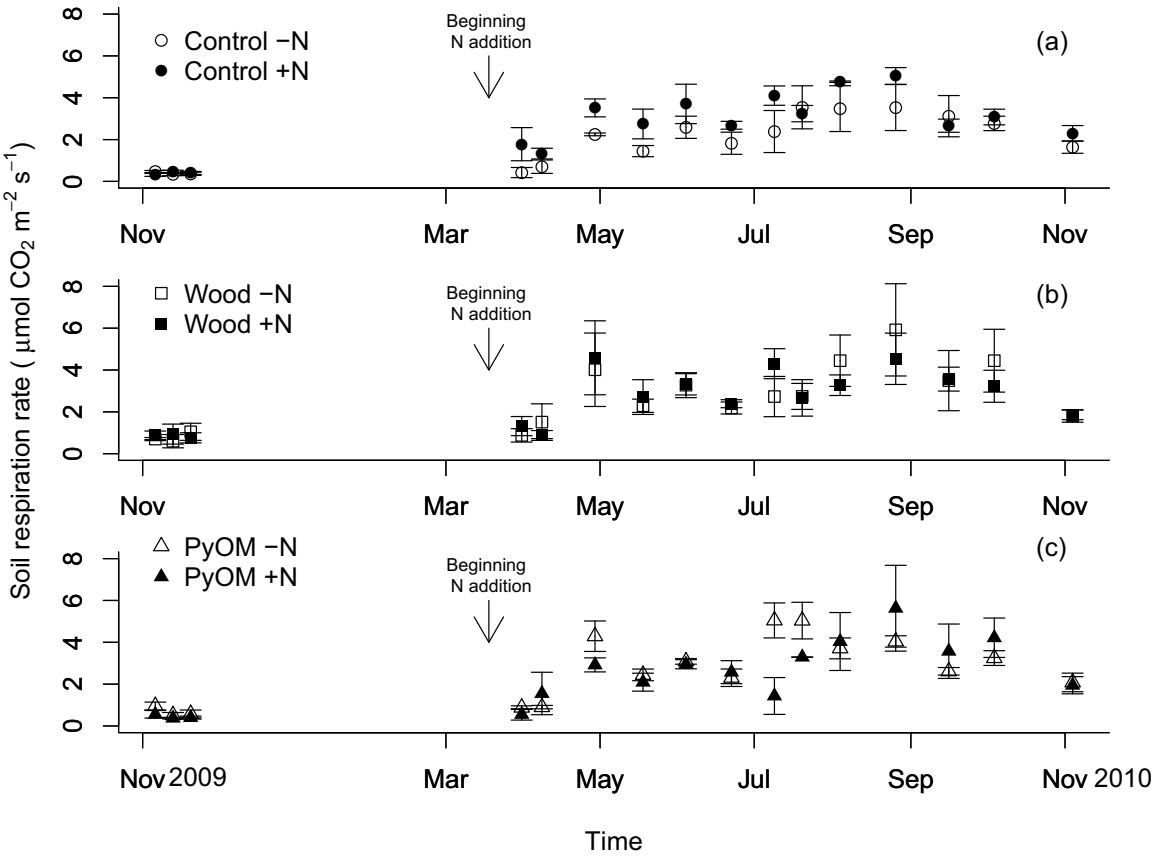

Control −N
Control +N
Beginning
N addition
(a)
Wood −N
Wood +N
(b)
PyOM −N
PyOM +N
(c)
Soil respiration rate (µmol CO2 m−2 s−1)
0 2 4 6 8
Nov 2009
Mar
May
Jul
Sep
Nov 2010
Time

Figure 2

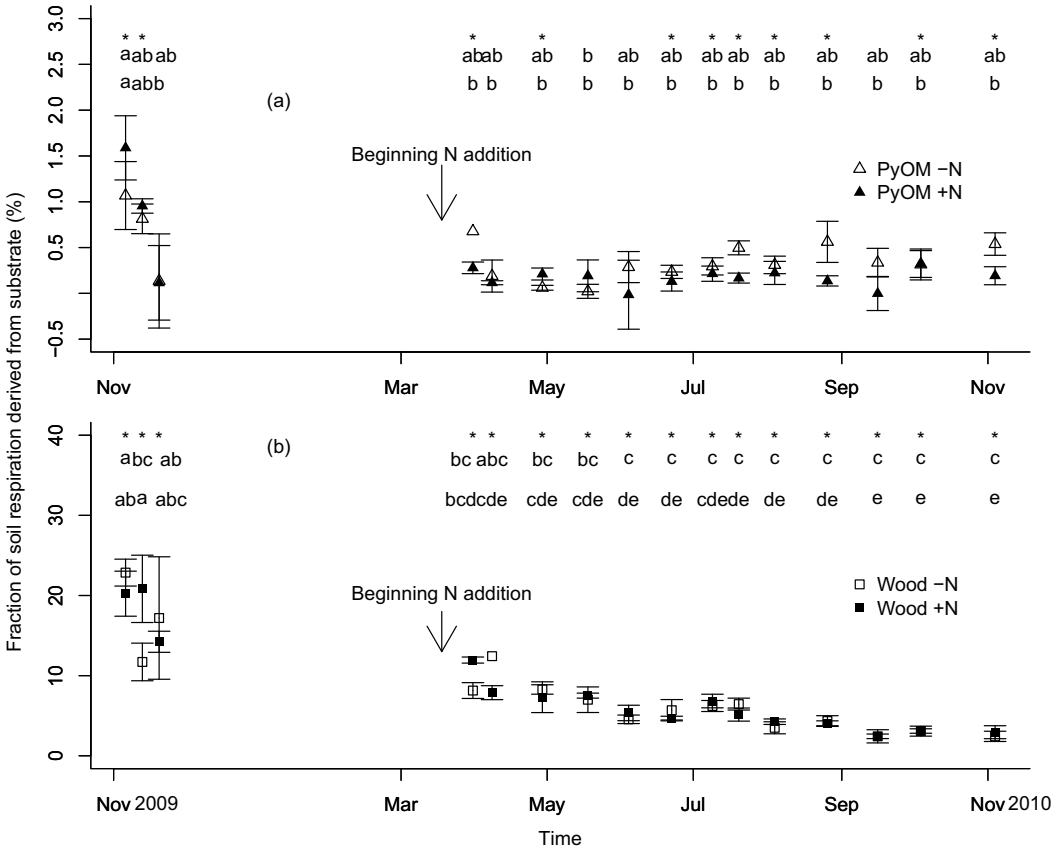

Fraction of soil respiration derived from substrate (%)
(a)
(b)
Beginning N addition
Beginning N addition
PyOM −N
PyOM +N
Wood −N
Wood +N
Nov 2009
Mar
May
Jul
Sep
Nov 2010
Time

Figure 3

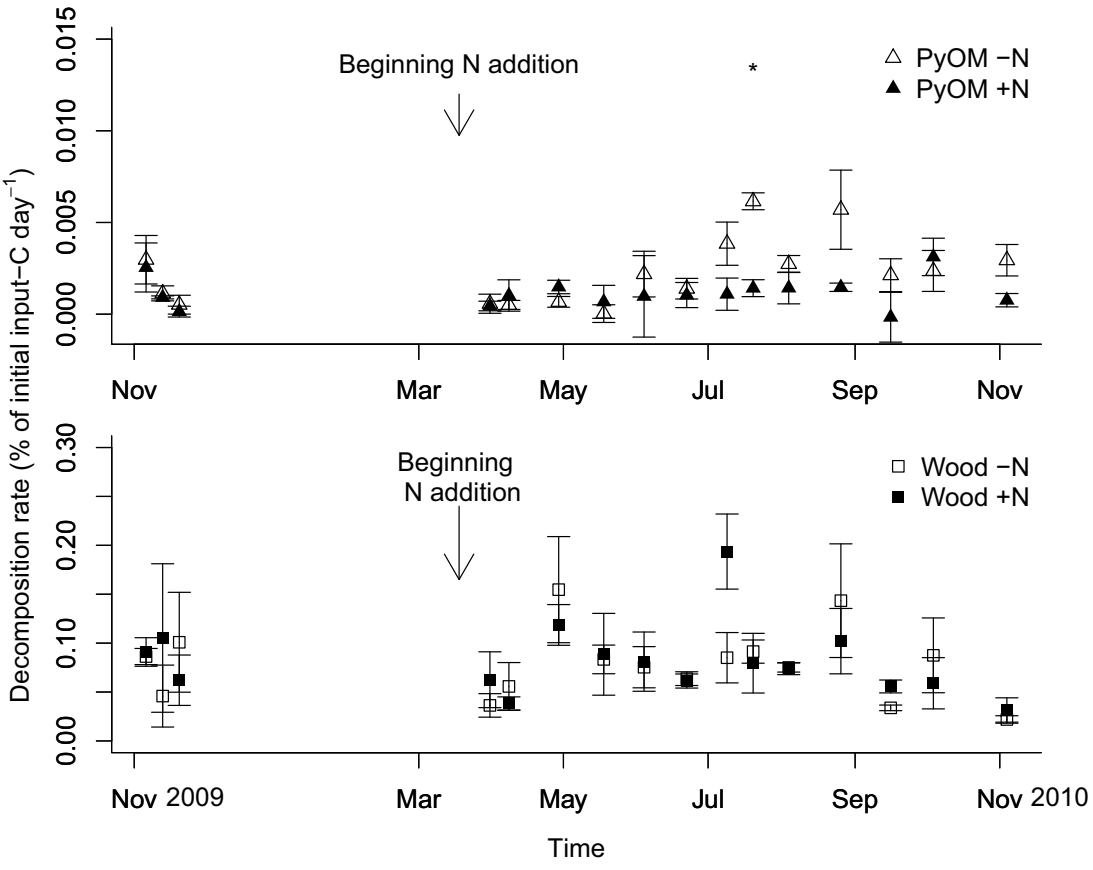

Beginning N addition
PyOM −N
PyOM +N
Beginning
N addition
Wood −N
Wood +N
Decomposition rate (% of initial input−C day^{-1})
0.000
0.005
0.010
0.015
0.00
0.10
0.20
0.30
Nov 2009
Mar
May
Jul
Sep
Nov 2010
Time

Figure 4

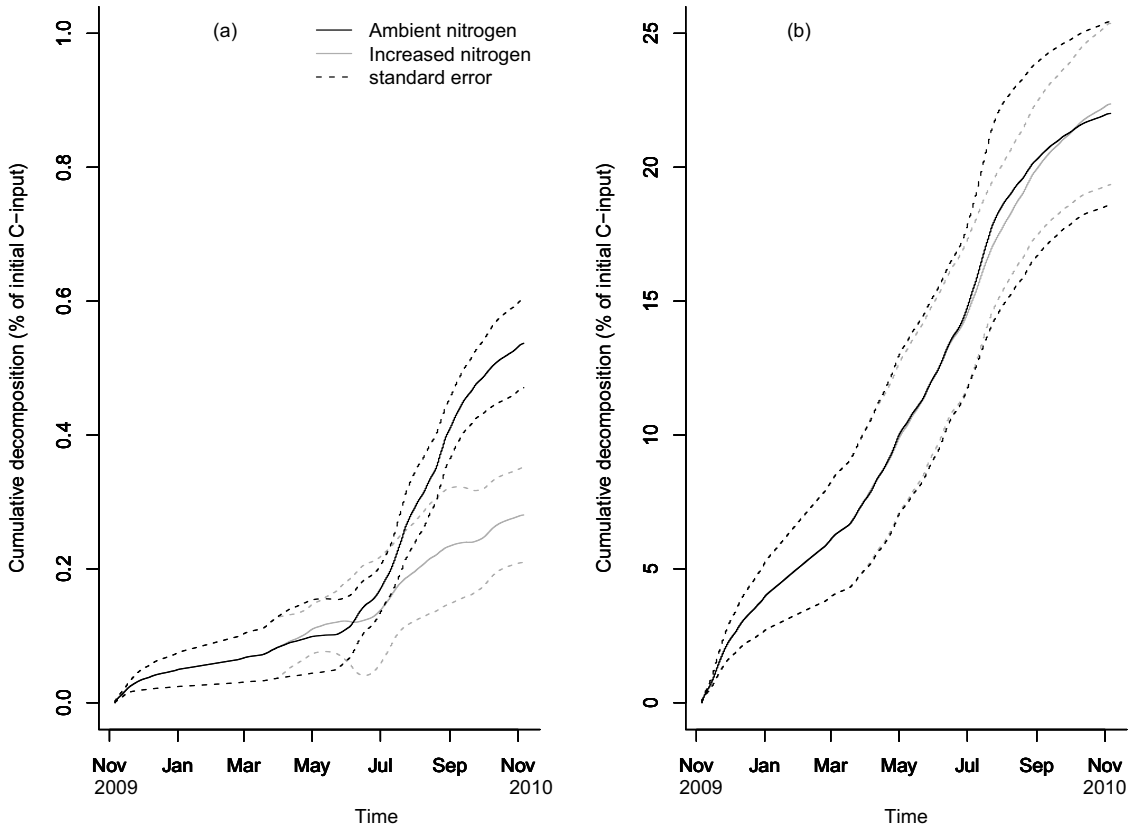

(a)
Ambient nitrogen
Increased nitrogen
standard error
Cumulative decomposition (% of initial C−input)
0.0
0.2
0.4
0.6
0.8
1.0
Nov
2009
Jan
Mar
May
Jul
Sep
Nov
2010
Time
(b)
Cumulative decomposition (% of initial C−input)
0
5
10
15
20
25
Nov
2009
Jan
Mar
May
Jul
Sep
Nov
2010
Time

Figure 5

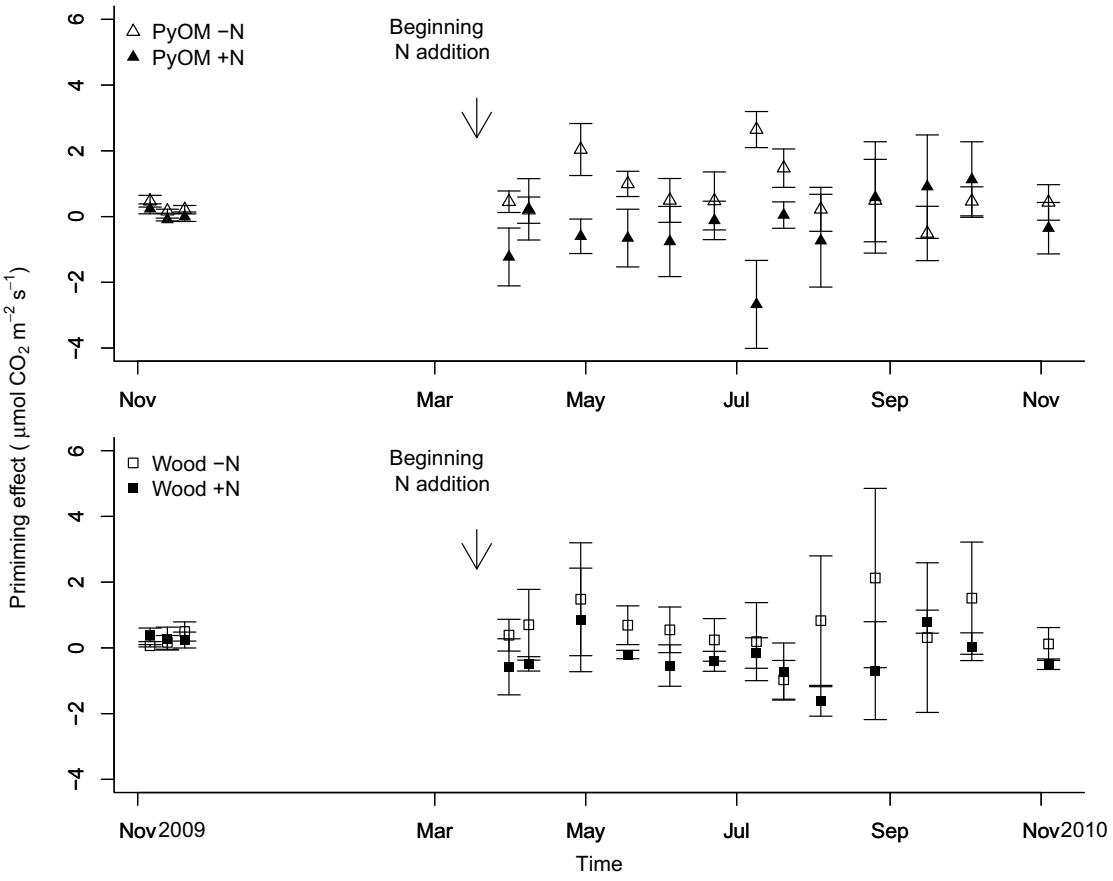

PyOM −N
PyOM +N
Beginning
N addition
Wood −N
Wood +N
Beginning
N addition
Primiming effect (µmol CO2 m−2 s−1)
6
4
2
0
−2
−4
Nov
Mar
May
Jul
Sep
Nov
Nov2009
Mar
May
Jul
Sep
Nov2010
Time

Figure 6

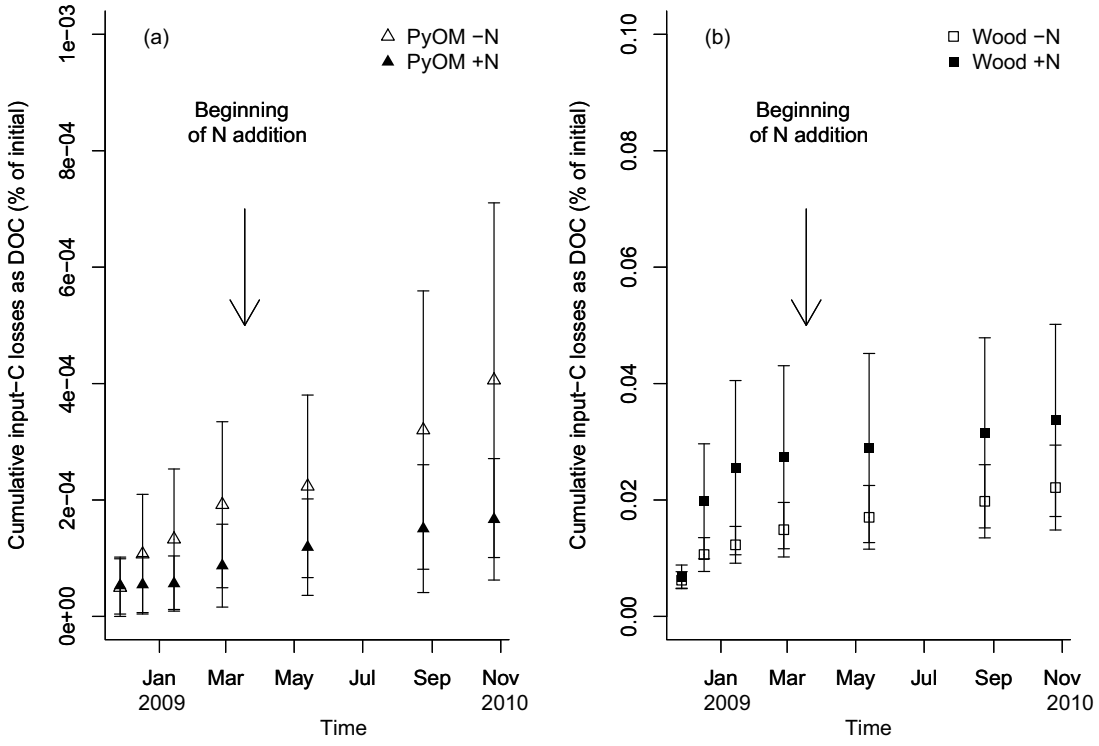

(a)
PyOM −N
PyOM +N
Beginning
of N addition
Cumulative input−C losses as DOC (% of initial)
0e+00
2e−04
4e−04
6e−04
8e−04
1e−03
Jan
2009
Mar
May
Jul
Sep
Nov
2010
Time
(b)
Wood −N
Wood +N
Beginning
of N addition
Cumulative input−C losses as DOC (% of initial)
0.00
0.02
0.04
0.06
0.08
0.10
Jan
2009
Mar
May
Jul
Sep
Nov
2010
Time

Figure 7

2 Manuscript II

Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil

Bernardo Maestrini, Anke M. Herrmann, Paolo Nannipieri, Michael W.I. Schmidt, Samuel Abiven

Article published in *Soil Biology and Biogeochemistry* 69:291-301, 2014.

Candidate contribution: The candidate conceived the experimental set-up, realized it, carried out measurement, data analysis and writing of the article.

Soil Biology & Biochemistry 69 (2014) 291–301

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil

Bernardo Maestrini [a], Anke M. Herrmann [b], Paolo Nannipieri [c], Michael W.I. Schmidt [a], Samuel Abiven [a,*]

[a] University of Zurich, Department of Geography, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[b] Swedish University of Agricultural Sciences, Uppsala BioCentre, Department of Chemistry, Box 7015, 750 07 Uppsala, Sweden
[c] University of Florence, Department of Plant, Soil and Environmental Sciences, Piazzale delle Cascine 18, 50144 Florence, Italy

ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form
30 October 2013
Accepted 13 November 2013
Available online 3 December 2013

Keywords:
PyOM
Biochar
Priming effect
Gross N mineralization
Net N mineralization
Protease
Phenol-oxidase

ABSTRACT

Pyrogenic organic matter (PyOM) is considered as a technique to improve soil fertility and store carbon (C) in soil. However, little is known regarding soil organic C and nitrogen (N) mineralization in PyOM-amended soils. To investigate the relationship between the C and N mineralization rates and the possible consequences in terms of C storage and N availability, we incubated ryegrass-derived PyOM (pyrolyzed at 450 °C) enriched in ^{13}C (4.33 atom %) in a forest Cambisol for 158 days with and without mineral N addition. We determined PyOM and native soil organic C mineralization, NH_4^+ and NO_3^- contents in the soil, gross N mineralization, phenol-oxidase and protease activities, and microbial biomass throughout the incubation experiment and the incorporation of PyOM in microbial biomass at the end of the experiment (158 days). We determined that 4.3% of the initial PyOM-C was mineralized after 158 days. Moreover, PyOM induced a strongly positive priming effect within the first 18 days; a negative priming effect was observed from Days 18 to 158. The initial increase in organic matter mineralization corresponded to a higher gross N mineralization and NH_4^+ content in the PyOM-treated soil than in the untreated soil. Ammonium was rapidly transformed into nitrate and stored in this form until the end of the experiment. We conclude that the presence of PyOM affected the mineralization pattern of native soil organic matter mineralization and increased mineral N content, while N addition did not influence PyOM or soil organic matter mineralization.

1. Introduction

Pyrogenic organic matter (PyOM), the product of incomplete combustion of biomass (Goldberg, 1985), plays an important role in the terrestrial C cycle because it can constitute up to 45% of soil organic carbon (Schmidt et al., 1999). PyOM has a turnover time of several centuries (Singh et al., 2012), a magnitude longer than any other class of soil organic compounds (Schmidt et al., 2011). Despite several recent developments in the assessment of PyOM stability (Bruun et al., 2008; Major et al., 2010; Santos et al., 2012), many uncertainties remain regarding its fate in the soil. In particular, little is known concerning the interaction between PyOM and the mineralization of native soil organic matter. Understanding this interaction is crucial for assessing the effect of PyOM on the soil C cycle because it may significantly modify the long-term C balance (Woolf and Lehmann, 2012). We here define the *priming effect* to be the change in the native organic matter mineralization rate due to the addition of an organic substrate (Bingeman et al., 1953). Specifically, we used the term positive priming effect when mineralization of the native organic matter is increased and negative priming effect when mineralization is decreased. PyOM has been observed in previous studies either to induce a positive priming effect (Wardle et al., 2008; Major et al., 2010; Novak et al., 2010; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011), a negative priming effect (Liang et al., 2010; Cross and Sohi, 2011; Jones et al., 2011), or no priming effect (Kuzyakov et al., 2009; Abiven and Andreoli, 2010; Cross and Sohi, 2011; Santos et al., 2012).

Changes in N mineralization were often found to follow C fluxes (Booth et al., 2005; Herrmann and Witter, 2008) because they are bound in the same organic compound. In fact, as for soil organic C mineralization, PyOM was found to exert a broad range of effects on the N cycle. This variability results from the differences in PyOM feedstock, pyrolysis temperature, and soil characteristics. Nelissen et al. (2012) found that a C-rich maize-derived PyOM increased

* Corresponding author. Tel.: +41 (0)44 635 51 83.
E-mail addresses: samuel.abiven@geo.uzh.ch, abiven@geo.unizh.ch (S. Abiven).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.11.013

gross short-term N mineralization in loamy soil. They suggested that microbes were "mining" soil organic matter to acquire N (Craine et al., 2007). DeLuca et al. (2002, 2006) observed that PyOM produced during wildfires increased nitrification in boreal and temperate forests and explained this as the result of sorption of phenols, which are known for being nitrification inhibitors, on PyOM surfaces (DeLuca and Sala, 2006; Ball et al., 2010). Moreover, Wang et al. (2012) observed an increase in nitrate content in a fertilized plot one year after the addition of rice husk-derived PyOM. Across three different soil types, Kolb et al. (2009) found that increasing the rate of PyOM addition, derived from a mix of manure and wood, reduced the amount of available N because of increasing microbial N demand. A similar conclusion was drawn using pecan-shell derived PyOM by Novak et al. (2010), while Bruun et al. (2012) found a relation between pyrolysis duration and the C:N ratio of the resulting PyOM, which was in turn affecting the quantity of N immobilized in the soil amended with PyOM. In contrast, no PyOM effect on the N cycle was observed by Zavalloni et al. (2011) and Zhang et al. (2011) using wood-derived PyOM and wheat straw-derived PyOM, respectively.

While many studies investigated the PyOM effects on mineral N, very little is known about the effect of mineral N on PyOM decomposition. Santos et al. (2012) found no effect of N addition on PyOM mineralization. However, Maestrini et al. (personal communication) found a decrease in PyOM mineralization. We hypothesized that N addition may decrease the PyOM decomposition because increased N deposition depresses the activity of phenol-oxidase (Sinsabaugh et al., 2002; Grandy et al., 2008), which is responsible for the decomposition of aromatic compounds. Moreover, we hypothesized that increased N availability will decrease microbial decomposition of the more recalcitrant fraction of PyOM, which is generally thought to be more rich in N, as proposed by the *nitrogen mining theory* (Craine et al., 2007). Similarly, Brodowski et al. (2005) suggested that microbes may decompose PyOM to have access to the N adsorbed on their surfaces. Changes in N fluxes due to increased microbial decomposition may be related to microbial biomass dynamics and thus can give an indication of both PyOM-C and mineral N stored by soil microflora (Nannipieri and Eldor, 2009).

To our knowledge, this study was the first to couple C fluxes and gross N mineralization in a PyOM-amended soil. The present paper is aimed to investigate if PyOM affects organic matter mineralization and if changes in C fluxes due to priming are reflected in N mineralization. We also hypothesize that N addition may reduce PyOM decomposition. To investigate the mechanisms responsible for the alteration of C and N fluxes, we used a holistic approach: we divided the system into pools (native soil organic matter, PyOM, microbial biomass, and mineral N) and related the C and N fluxes to the changes in the size of the pools and to the activity of enzymes targeting aromatic molecules, such as PyOM (phenol-oxidase) and N-rich compounds (protease). We believe that the holistic approach is the most efficient and well-adapted method for studying soil functionality compared to approaches based on the inference of C and N dynamics from microbial taxonomy and functional characterization (Nannipieri et al., 2003).

We incubated ^{13}C-labeled PyOM (4.33 atom %) for 158 days in a mineral forest soil with and without mineral N addition. We measured SOC mineralization, gross N mineralization, NH_4^+ and NO_3^- content, incorporation of PyOM derived C into microbial biomass and potential enzymatic activity of phenol-oxidase and protease over the course of the period.

Our research questions were as follows: (i) does ryegrass-derived PyOM increase native soil organic matter mineralization, gross N mineralization and net nitrification in a Cambisol? (ii) if so, can these changes be explained by the phenol oxidase and protease activity and microbial biomass-C and N?; (iii) does N addition affect mineralization of ryegrass-derived PyOM?.

2. Materials and methods

2.1. PyOM characteristics

Two different sets of ryegrass (*Lolium perenne* L.) were grown under controlled conditions in labeling growth-chambers. One set was grown under an atmosphere enriched in $^{13}C-CO_2$ (6 atom %); the other was grown under an ambient atmosphere. Edaphic, light, and air temperature conditions were identical for the two setups. Ryegrass was harvested after 1 month in both cases.

Labeled and not labeled grasses were pyrolyzed in a quartz tube oven (Montanaro manufacturer, Glattbrugg, CH) at 450 °C under a N_2 stream of 1 L min^{-1} (equivalent to 0.45 times the volume of the oven per minute) for 4 h as described in Hammes et al. (2006). The recovery of PyOM after pyrolysis was approximately 33% (weight %) of the initial material. Characteristics of the ^{13}C-labeled PyOM are summarized in Table 1. The set of ryegrass grown under enriched $^{13}C-CO_2$ conditions had slightly higher C and N contents (30 vs. 34% C and 3.2 vs. 3.6% N, $p < 0.05$, t-test, $n = 4$), compared to the one grown under unlabeled conditions. However, the C:N ratios of the two sets did not significantly differ. The PyOM had a low C content (34%) and a high O (28.0%) and ash contents (53% residual after ignition at 550 °C for 2 h). The H:C atomic ratio was 0.67 ± 0.02, which is similar to values reported by Hammes et al. (2006) and Keiluweit et al. (2010) for grass-derived PyOM. This indicates that the PyOM had a relatively low C content due to a high content in microelements (resulting in high ash content). However, the aromaticity level, indicated by the H:C ratio, did not differ from other grass-derived PyOM. The low C content of our PyOM agrees with findings from Knicker (2010), who also observed a C content of 30% for ryegrass-derived PyOM due to the low thermal stability of cellulose, a major component of grass, as also observed by Chatterjee et al. (2012). Our PyOM was characterized by a narrow C:N ratio, smaller than 10, and a very high ash content (Table 1), values similar to C:N ratio and ash content of PyOM derived from ryegrass obtained in another study (Knicker, 2010) this indicates that characteristics of ryegrass-derived PyOM may be similar. In contrast Keiluweit et al. (2010), using a different grass species, found a higher value. The main explanation for the low value is the higher level of N incorporation in the pyrolysis products compared to C. In the study from Knicker (2010), N was observed to occur mostly in heterocyclic forms, like pyrroles. High ash content may also result from low thermal stability of cellulose.

The ^{13}C-labeled PyOM had a ^{13}C value of 4.33 atom % (Table 1); we have assumed that ^{13}C was uniformly distributed within the plant because it was grown in an atmosphere enriched in $^{13}C-CO_2$ from the first emergence of a leaf.

2.2. Incubation setup

We sampled the top 10 cm of a Cambisol in a clearance of a temperate forest on Laegeren Mountain (NW of Zurich, Swiss Plateau, 800 m asl., Ruehr et al., 2009). The characteristics of the soil are summarized in Table 1. The soil was sieved fresh through a 2-mm mesh. The equivalent of 80 g dry soil was weighed into crystallizing dishes (Duran, Germany) 70 mm in diameter and placed inside a sealed 1.8-L jar (Korken, IKEA). In the vessels the soil had a bulk density of 0.7 g cm^{-3}, and no effect of PyOM was observed on bulk density. The soil was pre-incubated at 27 °C for 23 days prior to the beginning of the incubation. The temperature and soil moisture were kept constant throughout the entire

Table 1
Characteristics of the soil and PyOM, values are the average of 4 replicates ± standard error.

	pH	Texture (mass %)			Bulk density (in the field)	C content	N content	C/N	Ashes ($n = 2$)	^{15}N	^{13}C	NO_3^-	NH_4^+
		Sand	Silt	Clay	g cm^{-3}	mg C g^{-1} dry soil	mg N g^{-1} dry soil	(w/w)	(mg g^{-1})	(atom %)		μg N g^{-1} soil	
Soil	5.72 ± 0.04	45.5 ± 3.5	24.2 ± 4.4	31.5 ± 2.4	1.4	35.6 ± 0.01	2.93 ± 0.03	12.1		0.3664 ± 0.0001	1.0761 ± 0.0001	56.85 ± 0.76	1.53 ± 0.65
						mg C g^{-1} PyOM	mg N g^{-1} PyOM					mg N g^{-1} PyOM	
PyOM	10.02 ± 0.01					344 ± 3	36.87 ± 0.06	9.3	530.4 ± 2	0.36888 ± 0.00005	4.33 ± 0.01	0.05 ± 0.02	0.4 ± 0.1

incubation period at 27 °C and 70% of the water holding capacity, respectively. The soil moisture content was periodically adjusted (fluctuations in the soil moisture content were therefore generally lower than 1% weight). A bottle containing 20 mL of water was placed inside the jar to maintain the humidity saturation of the air. The incubation consisted of a 2 × 2 factorial experiment with the following treatments: soil control, soil + PyOM, soil + mineral N, soil + PyOM + mineral N. Nitrogen treatment corresponds to an addition of 25 μg N–NH_4NO_3 g^{-1} dry soil at the beginning of the incubation. This quantity is equivalent (considering the top 15 cm of the soil) to 53 kg N ha^{-1}, which is in the range applied yearly in two well-known field experiments on N deposition (Aber et al., 1998; Egli et al., 1998). N was added from an aqueous solution containing approximately 181 mg N–NH_4NO_3 l^{-1}. We added an equivalent amount of water to the control soils.

At the beginning of the incubation we added the equivalent of 13 mg PyOM g^{-1} dry soil to PyOM-treated vessels and all samples were mixed thoroughly. This quantity was equivalent to an addition rate of 27 t ha^{-1}, considering an application to the first 15 cm of the soil and a bulk density of 1.4 g cm^{-3}. Unlabeled PyOM was added to vessels to be extracted after 4, 18, 46 and 88 days whereas ^{13}C-labeled PyOM was added to the vessels to be extracted on the last sampling date, i.e., after 158 days. On Days 4, 18, 46, 88, and 158 after incubation started, soils were sampled for analysis of mineral N content (NH_4^+ and NO_3^-), gross N mineralization (see Section 2.4) and microbial biomass (see Section 2.5). Phenol-oxidase and protease activities and soil pH were measured on Days 4, 46, and 158 (see Section 2.5).

2.3. CO_2 efflux and partitioning

CO_2 efflux and ^{13}C–CO_2 were monitored throughout the incubation experiment. CO_2 efflux from the soil was trapped in bottles containing 20 mL of 1 M NaOH and subsequently placed in the jars. The amount of CO_2 trapped as sodium carbonate (Na_2CO_3) was estimated by measuring the decrease in conductivity using the linear model described by Wollum and Gomez (1987) and recently applied by Abiven and Andreoli (2010). A set of blanks ($n = 4$) was also measured to account for the CO_2 initially present in the container; both the quantity of CO_2 emitted and the isotopic signal were accordingly corrected. The jars were opened only at the reported sampling dates. After measuring the conductivity, the NaOH vials were removed and substituted with new ones so that on each date we could measure the cumulative CO_2 emitted from the sample.

Briefly, the ^{13}C–CO_2 was measured by precipitating trapped CO_2 with $BaCl_2$ as described in Gaillard et al. (2003). An aliquot of 5 mL of NaOH solution was added to 10 mL 1 M $BaCl_2$, and subsequently filtered (<0.45 μm cellulose acetate filter paper, *GVS*, *Bologna, Italy*). The precipitates remaining on the filter were then dried, crushed with a spatula, and an aliquot of approximately 5 mg was used for the ^{13}C analysis using an isotope mass ratio spectrometer (Delta S, Thermo Finnigan, USA). To partition the origin of the trapped CO_2 between the native soil organic matter and PyOM, we used a two-source isotope mixing model equation:

$$f = 1 - \left({}^{13}C_{mix} - {}^{13}C_{PyOM}\right) / \left({}^{13}C_{control} - {}^{13}C_{PyOM}\right), \quad (1)$$

where f is the fraction of CO_2 derived from PyOM, $^{13}C_{mix}$ is the ^{13}C content of the trapped CO_2, $^{13}C_{PyOM}$ represents the ^{13}C content of PyOM, i.e., 4.33%, and $^{13}C_{control}$ is the isotopic signature of soil CO_2 in the corresponding control treatment.

The priming effect induced by PyOM on native soil organic C mineralization was calculated using:

$$PE = (SR_{PyOM}*(1-f) - SR_{control})/SR_{control}*100, \quad (2)$$

where SR_{PyOM} and $SR_{control}$ are soil respiration in PyOM and the control soil, respectively, and f is the fraction of soil respiration derived from PyOM mineralization using Equation (1). In Equation (2), PE is expressed as the percentage of soil respiration in the control treatment. To calculate mean residence time based on the cumulative PyOM mineralization data, we used a two-pool parallel exponential decay model (Manzoni and Porporato, 2009; Minderman, 1968) as follows:

$$C_t = C_0*fr*\exp(-k_1*t) + C_0*(1-fr)*\exp(-k_2*t), \quad (3)$$

where C_t is PyOM at time t and C_0 is the initial quantity of PyOM added. The fitted parameters were fr, k_1 and k_2, which represent the fast pool fraction (dimensionless), and the PyOM mineralization rate, expressed as % of PyOM-C lost per day, of the fast (k_1) and slow (k_2) pools, respectively; t is the time in years. Parameters where refined by successive iterations to minimize the residual sum-of-squares. From the mineralization rates (k_1 and k_2) we derived the mean residence time (MRT) of the corresponding pool as:

$$MRT = 1/k_{1,2}, \quad (4)$$

where k_1 corresponds to the MRT of the fast turning pool and k_2 refers to the slow turning pool.

2.4. Mineral N content and gross N mineralization

Total mineral N was extracted using a 1 M KCl solution (1 h of shaking, 180 rpm, 1:4 soil:solution ratio). Nitrate and ammonium concentrations were determined using spectrophotometry (San^{++}, Skalar, Netherlands). To measure gross N mineralization, we used the ^{15}N pool isotope dilution technique (Murphy et al., 2003). 40 g of dry soils were amended with 2 mL of a 100 mg N–$(NH_4)_2SO_4$ l^{-1} solution labeled with ^{15}N (2.7 atom %), giving a 5 μg N–$(NH_4)_2SO_4$ g^{-1} dry soil. The solution was added drop-wise onto the soil surface after which the soil samples were thoroughly mixed to homogenize added N distribution. After 4, 24, and 72 h, an aliquot of 10 g of fresh soil was extracted (using 1 M KCl) and measured for total NH_4^+ content and ^{15}N–NH_4^+ using the diffusion technique described by Herrmann et al. (2007). Briefly, 15 mL of KCl soil extract was filled into a 20 mL scintillation vial and approximately 200 mg MgO was added to generate NH_3 for the determination of the atom % ^{15}N of the NH_4^+ pool. The evolved NH_3 was trapped onto an acidified paper disk which was placed between a double layer of polytetrafluoroethylene (PTFE) tape and stretched over the top of the scintillation vials which were then capped. All samples were gently shaken for 72 h to transform NH_4^+ into NH_3. To prevent the introduction of sulfur in the isotopic ratio mass spectrometer the method was modified according to Schleppi et al. (2006), i.e., using citric acid instead of sulfuric acid. The isotopic signature of the ^{15}N–NH_4^+ trapped on the acid filters was then measured using an isotope ratio mass spectrometer (Delta S, Thermo Finnigan, USA). To calculate gross N mineralization fluxes, we used the formula from Khirkham and Bartholomew (1949), as reported in Smith et al. (1992):

$$\text{gross mineralization} = \{[(AT_1 - AT_2)/\Delta t]*[\log(AL_1*AT_2)/\log(AL_2*AT_1)]\}/\log(AT_1/AT_2) \quad (5)$$

where AT is the total amount of NH_4^+ (μg N g^{-1} dry soil), AL is the amount of recovered ^{15}N–NH_4^+ (μg N g^{-1} dry soil), and Δt is the time between subsequent extractions (hours). In our study two times intervals were considered: (i) 20 h, i.e. KCl extraction 4 and 24 h after ^{15}N addition, and (ii) 48 h, i.e. KCl extraction 24 and the 72 h after ^{15}N addition. The subscripts indicate the extraction time. Estimated gross N mineralization rates were similar in the two time intervals, i.e., 2–24 and 24–72 h (paired t-test, $p > 0.05$). Therefore, the assumption of zero-order kinetics of gross N mineralization was met in the present experiment and we calculated an average value of gross N mineralization across the two time intervals.

2.5. Enzyme activities, pH and microbial biomass

Protease activity was measured using casein as substrate as described by Alef and Nannipieri (1995); phenol-oxidase was measured using a di-phenol (3,4-diidrossi-l-fenilalanina, also named L-DOPA) substrate as described by Carreiro et al. (2000). Although other substrates have been proposed to assess phenol-oxidase, e.g., guaiacol, a mono-phenol (Nannipieri et al., 1991), and others (Baldrian, 2006), we used L-DOPA because it is the most adopted substrate in environmental studies and has a very high sensitivity (Sinsabaugh, 2010). Both reactions were performed at pH 8.2. Soil pH was measured in a 1:5 soil:water (fresh weight:weight) mixture after shaking and subsequent sedimentation for 12 h.

Microbial C and N were measured by the fumigation–extraction method (Vance et al., 1987) in which 10 g of fresh soil were fumigated with alcohol free chloroform in a desiccator for 24 h. The samples (both fumigated and non-fumigated) were then extracted using a 1 M KCl solution. The total organic C (TOC) and total N in the fumigated extracts were analyzed using a TOC-TN analyzer (TOC-V, Shimadzu Corporation, Japan). Microbial C and N concentrations were determined by subtracting C and N in the non-fumigated treatment from C and N in the fumigated treatment and multiplying by a factor of 2.64 (Vance et al., 1987).

We also determined the fraction of the microbial biomass derived from labeled PyOM on the last sampling date (158 days). An aliquot of 10 mL from the fumigated and non-fumigated extracts was freeze-dried and the resulting material was measured for ^{13}C content with an isotope mass ratio spectrometer (Delta S, Thermo-Finnigan, *USA*). The ^{13}C signature of the microbial biomass was then estimated using Equation (5) (Dawson et al., 2002):

$$^{13}Cmb = \left(\left(^{13}C_{fum}*C_{fum}\right) - \left(^{13}C_{non-fum}*C_{non-fum}\right)\right) \Big/ \left(C_{fum} - C_{non-fum}\right), \quad (6)$$

where C_{fum} and $C_{non\text{-}fum}$ are the amounts of C extracted from the fumigated and non-fumigated samples (μg C g^{-1} dry soil) and $^{13}C_{fum}$ and $^{13}C_{non\text{-}fum}$ were the ^{13}C contents of the fumigated and non-fumigated extracts (atom %). To quantify the portion of microbial C derived from the added PyOM, we used Equation (1), substituting $^{13}C_{mix}$ with $^{13}C_{mb}$ and ^{13}C with the $^{13}C_{mb}$ in the control treatment. Liang et al. (2010) pointed out that the use of chloroform fumigation extraction in soils rich in PyOM, might underestimate microbial biomass due to the readsorption of lysed cells on PyOM walls. Nevertheless, we believe that even if such underestimation may be pronounced, it is of minor importance in our experiment, as the ratio between the PyOM-C and soil organic carbon ratio was 7 times lower than reported in Liang et al. (2010). Therefore, we expect that the underestimation of the microbial biomass due to PyOM sorption of lysed cells will have a minor effect.

2.6. Statistical analyses

The effects of PyOM and N addition were tested using a two-way analysis of variance (ANOVA) for all variables, except for CO_2 effluxes, where repeated measures two-way ANOVA was adopted.

Two-way ANOVA were also performed separately for each sampling date. When data were not normally distributed according to the Shapiro normality test ($p > 0.05$), the data were log transformed. The Kruskal–Wallis test was adopted instead of ANOVA, if also log-transformed data were not normally distributed. When time was a significant factor, we performed a Tukey-post-hoc test to determine which sampling dates were significantly different. All computations were performed using the statistical software *R*. The "agricolae" package was used to perform the Tukey test; the "ezANOVA" package was used for the repeated measures ANOVA.

3. Results

3.1. Soil respiration, native and pyrogenic organic matter (PyOM) mineralization

Soil respiration was significantly influenced by time ($p < 0.05$), the presence of PyOM ($p < 0.05$) and the interactions between PyOM and time ($p < 0.05$). Particularly, the presence of PyOM increased the total soil respiration within the first 18 days and decreased it afterwards (Fig. 1a). Neither PyOM nor N addition altered the total net cumulative soil respiration over 158 days of incubation (Table 2). After 158 days, PyOM-C losses as CO_2 were 4.3 ± 0.1 and 4.4 ± 0.1% of the added PyOM-C, with and without N addition, respectively. Most of the PyOM mineralization occurred within the first 4 days (approximately 2.9% of the initial PyOM-C). The PyOM mineralization was not influenced by N addition at any sampling date during the incubation; the cumulative mineralization at the end of the experiment was also not affected (Table 2). We fitted a two-pool exponential decay model to our PyOM mineralization data (Equation (3)) and found no significant differences in the mean residence times (Table 3) between N addition treatments. The fast pool represented 3.3% of the initial PyOM-C and had an MRT of 2 days; the slow pool had an MRT of 40 years (Table 3). Over the 158-day period, the presence of PyOM inhibited cumulative native organic matter mineralization ($p < 0.05$, Table 2), i.e., it induced a negative priming effect equivalent to 10.09 ± 3.08 or 13.53 ± 3.11% of the soil respiration in the control treatment with or without N treatment, respectively. However, the priming effect direction changed over time. Within the first 18 days, PyOM induced a positive priming effect; a negative effect occurred from Day 18 to Day 158 (Kruskal-test or ANOVA test on individual dates, $p < 0.05$, Fig. 1c). The N addition did affect the priming effect.

3.2. Microbial biomass

Over the entire incubation period, the PyOM addition increased the microbial biomass C ($p < 0.05$, Fig. 1d) in comparison with control treatments. Within treatments, the microbial biomass C decreased over time (Tukey post-hoc test between dates, $p < 0.05$), while the N addition did not alter the microbial biomass C. The increase in soil microbial biomass C due to PyOM addition was only significantly different on Days 4, 18, and 88 ($p < 0.05$). We did not find an effect of PyOM or N addition on microbial biomass N and the

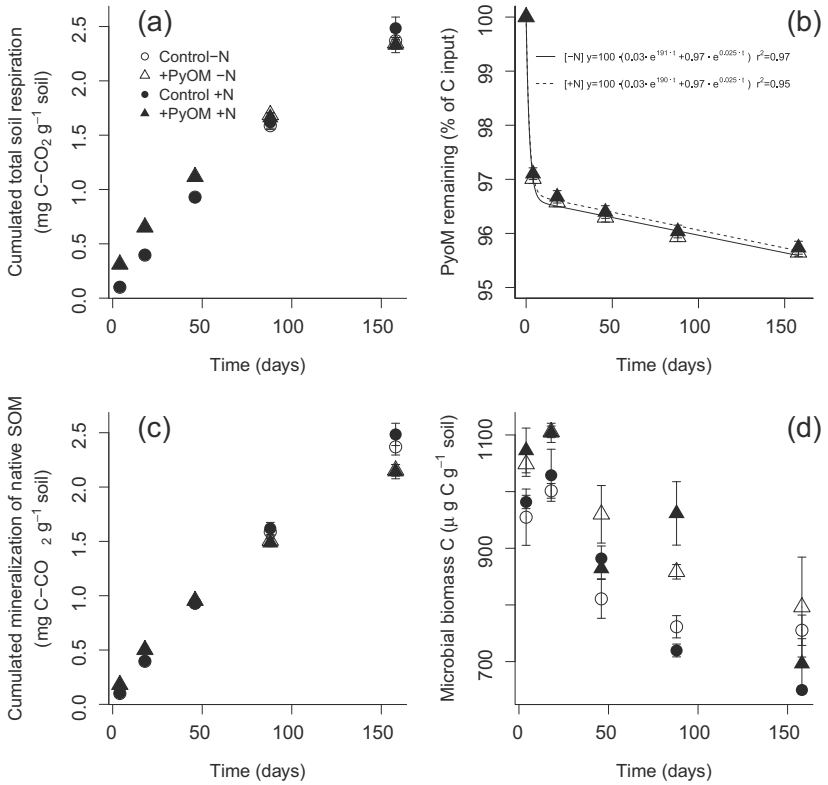


Fig. 1. Cumulative total soil respiration (a), PyOM remaining in the soil as measured and modeled according to Equation (3) (b), cumulative mineralization of native soil organic matter (c) and (d) microbial C dynamics throughout the incubation period. Full symbols represent the experiment without N addition treatment: empty symbols are for the N addition treatment. The circles represent the control treatments and triangles are for PyOM addition treatments. The dashed line represents the modeled PyOM mineralization with N treatment; the continuous line is for the PyOM mineralization without N treatment. In all figures, the error bars represent the standard error of the mean ($n = 4$).

Table 2
Total soil respiration, cumulative native organic matter mineralization, potential protease activity (substrate caseine), and cumulative PyOM mineralization. Values are average of four replicates ± standard error of the mean.

	Cumulated soil respiration (after 158 days)	Cumulated native organic matter decomposition (after 158 days)	Cumulative PyOM decomposition (after 158 days)	Mean protease activity	Fraction of microbial biomass C derived from PyOM (after 1 days)
	(mg C–CO_2 g^{-1} soil)	(mg C–CO_2 g^{-1} soil)	(% of initial input)	(μg tyrosine g^{-1} soil h^{-1})	%
Control -N	2.37 ± 0.08	2.37 ± 0.08		1.73 ± 0.12	
PyOM input -N	2.34 ± 0.05	2.15 ± 0.05	4.4 ± 0.18	1.76 ± 0.08	0.47 ± 0.02
Control + N	2.48 ± 0.10	2.48 ± 0.10		1.70 ± 0.07	
PyOM input + N	2.33 ± 0.07	2.14 ± 0.06	4.3 ± 0.1	1.92 ± 0.12	0.45 ± 0.03

microbial C:N ratio (Table 1, Supplementary material). The fraction of PyOM-derived C recovered in the microbial biomass after 158 days was 0.45 ± 0.03 and 0.47 ± 0.02% (*t*-test, $p < 0.05$, Table 2) with and without N addition, respectively, corresponding to 0.07 ± 0.01 and 0.08 ± 0.01% of the initially added PyOM-C, with no significant difference between the N addition treatments.

3.3. Nitrogen cycling

Mineral N content in the soil increased significantly after PyOM addition ($p < 0.05$), mineral N addition ($p < 0.05$) and over time ($p < 0.05$, Fig. 2a). PyOM increased the NH_4^+ content after 4 days (Kruskal-test, $p < 0.05$, Fig. 2b), while we found almost no NH_4^+ on Day 18 in both treatments. After 18 days, the NH_4^+ content increased again. However, we could not observe a clear trend in NH_4^+ content for all treatments. For individual dates, PyOM addition affected significantly the gross N mineralization on Days 4 ($p < 0.05$) and 158 (Kruskal test, $p < 0.05$). However, on sampling Days 18 and 46, the NH_4^+ contents in the extracts after 72, and sometimes even 24 h after ^{15}N addition, were extremely low. More specifically, NH_4^+ was not detectable in some PyOM-amended soils. Therefore, measurements from those dates are unreliable. This decrease of NH_4^+ from the mineral N pool in soil amended with PyOM may be directly related to PyOM sorption capacities (Jones et al., 2012). Overall net nitrification rates (Fig. 2d) were not affected by PyOM addition. However, it was affected by N addition ($p < 0.05$) and time ($p < 0.05$). For individual dates, N addition increased nitrification from Day 0 to Day 4 ($p < 0.05$), very likely a result of the nitrification of NH_4^+ added as NH_4NO_3 at the beginning of the experiment. In comparison, PyOM increased the net nitrification from Day 4 to 18 ($p < 0.05$). No differences in nitrification were observed after Day 18 due to the addition of PyOM or N.

3.4. Enzyme activities and soil pH

PyOM addition decreased the activity of phenol-oxidase ($p < 0.05$, Fig. 3) compared to the control treatment; we did not observe an N addition effect. In contrast no PyOM addition, N addition or time effects were observed on protease activity. PyOM addition significantly increased pH (Kruskal–Wallis test, $p < 0.05$, Fig. 1 Supplementary material) for the entire duration of the experiment. Soil pH decreased for all sampling dates for the PyOM treatment and only between the first and second sampling dates for the control treatment (pairwise Wilcox-test, $p < 0.05$). Moreover, for the first sampling date, the control with N had a lower pH than the control without N (Wilcox-test, $p < 0.05$). This could be attributed to the initial nitrification of the added NH_4^+.

Table 3
Mean residence time (MRT) calculated with the two-pool exponential decay model fitted to the mineralization dynamics corresponding to the treatments without and with N addition. Values are average of four replicates ± standard error.

	MRT labile	MRT resistant	Fast pool fraction
	(days)	(years)	(% of initial PyOM-C)
Without N	1.92 ± 0.03	40.35 ± 0.31	3.4 ± 0.1
With N	1.92 ± 0.02	39.79 ± 0.33	3.3 ± 0.1

4. Discussion

4.1. PyOM mineralization, soil respiration and phenol oxidase activity

After five months of incubation, 4.3% of PyOM-C was mineralized (Table 2). Our results are comparable to previous findings on ryegrass-derived PyOM decomposition from Hilscher et al. (2009). They found a decomposition ranging between 2.5 and 3.2% of the initial PyOM-C after 52 days, depending on the duration of the pyrolysis process, with longer durations delivering more resistant PyOM. Using our model (Fig. 1b) we found that 3.7% of PyOM-C was decomposed after 52 days. We believe that the difference between the two studies is due to the different edaphic conditions. Specifically, they incubated PyOM in a B horizon poorer in organic C (3.4 mg C g^{-1} soil), and most likely also less microbial biomass compared to our study. Also the PyOM characteristics may have played a role. In fact their PyOM was produced at a lower temperature and was characterized by a higher C:N ratio. Hamer et al. (2004) incubated ryegrass-derived PyOM and microbial inocula in quartz sand and found that only 0.8% of PyOM-C was decomposed after 60 days. This confirmed that soil characteristics, e.g., microbial structure, and aggregation play a crucial role in determining PyOM stability. We fitted a two-pool decomposition model to our PyOM mineralization data (Fig. 1b) and observed that PyOM had a fast pool with a turnover time of 2 days, equivalent to 3.3% of PyOM-C, and a slower pool, with a turnover time of 40 years (Table 3). These values are in agreement with previously reported PyOM turnover times determined from a meta-analysis for grass-derived PyOM in incubation studies by Singh et al. (2012). Several authors observed that pyrolysis may increase carbonate content of the pyrolysis product (Lehmann and Joseph, 2009). Therefore it is likely that part of the initial high PyOM-C losses derives from PyOM-inorganic C, i.e., carbonates (Jones et al., 2011; Bruun et al., 2013). The release of CO_2 from carbonates is also reflected in the pH decrease over time (Fig. 1, Supplementary materials), decreasing as PyOM-carbonates were consumed. Using the two-pool model, we predict that the quantity of PyOM remaining in the soil after 100 years (which is the minimum permanence requested by many C reduction schemes) will be 8% of the initial PyOM-C. Such a relatively fast decomposition rate would represent a challenge for the use of PyOM as a tool to store C in the soil. Nevertheless, caution is necessary when using exponential decomposition models to predict the long-term stability of PyOM. In fact, models calibrated on short-term experiments capture only the initial fast decomposition rate of PyOM and

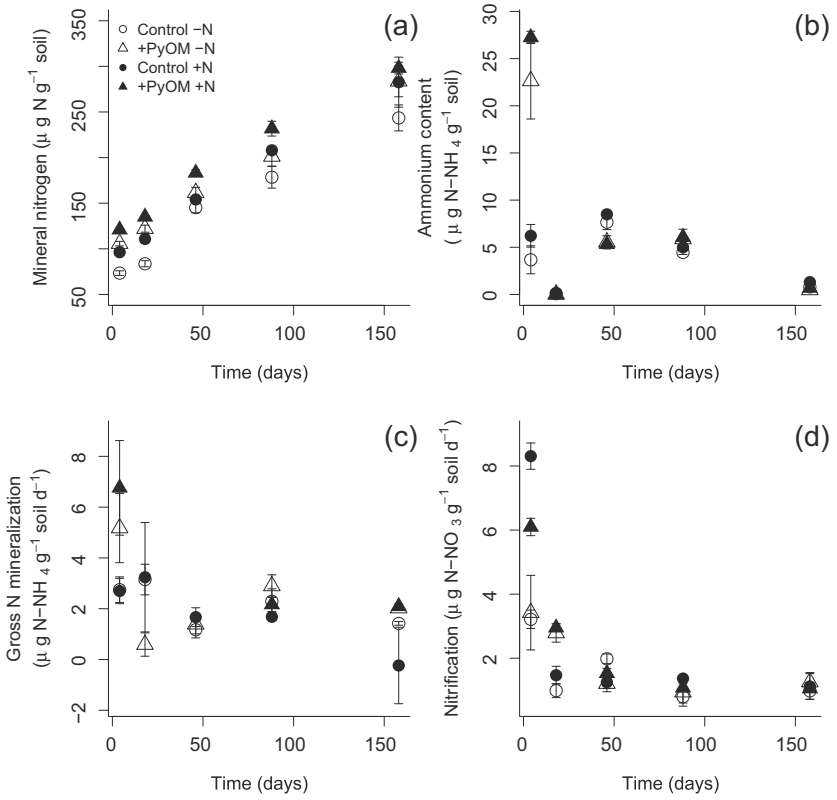


Fig. 2. Mineral N dynamics in the soil along the incubation period: (a) soil mineral N content, (b) soil NH_4^+ content (c) gross mineralization and, (d) nitrification. Full symbols represent without N addition treatment, empty symbols represent with N addition treatment, circles are for control treatments and triangles are for PyOM addition treatments. Error bars represent the standard error of the mean ($n = 4$). On sampling days 18 and 46, the measurement of gross N mineralization failed because the NH_4^+ contents in the extracts after 72, and sometimes even 24 h after ^{15}N addition, were extremely low, sometimes below the detection limit.

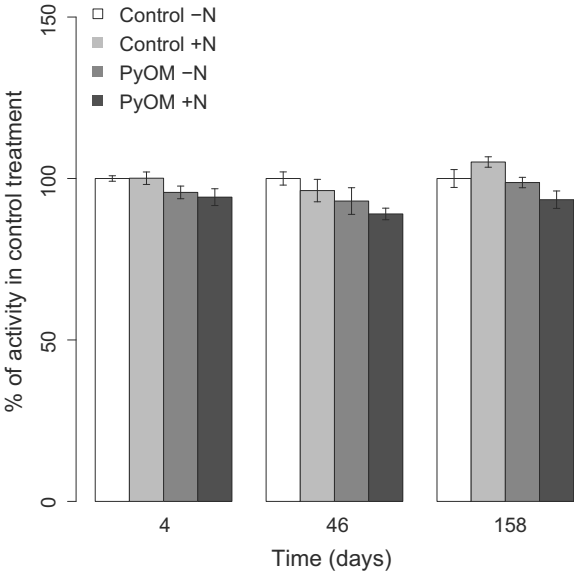


Fig. 3. Potential phenol-oxidase activity (using L-DOPA as substrate). Error bars represent the standard error of the mean ($n = 4$). Within each sampling date, the bars are in the following order: control without N addition, control with N addition, PyOM without N addition and PyOM with N addition.

therefore they may overestimate PyOM decomposition (Singh et al., 2012).

N addition did not affect PyOM-C losses over time, confirming previous findings by Santos et al. (2012). Because our soil was not N-limited and the net N mineralization was positive throughout the incubation period, it was unlikely that N addition would play an important role. Surprisingly, the activity of phenol-oxidase was inhibited by PyOM addition but not by N addition, which is not in agreement with previous observations that N addition may inhibit phenol-oxidase (Sinsabaugh, 2010). DeLuca et al. (2002) observed that PyOM has the capacity to absorb phenols. This may lead to a decrease in the concentration of the assay, resulting in a lower availability of the assay for the targeted enzymes and therefore in a decrease of enzymatic activity. A decrease in phenol oxidation due to sorption on mineral surfaces was also observed by Scott et al. (1983). It is important to consider that the assay method used in the present study (L-DOPA, an o-diphenol), although widely used, is only one of the plethora of assays that have been employed to measure phenol-oxidase activity. It is most likely that it does not cover the entire range of enzymes involved in the oxidation of phenols or related molecules, e.g., the aromatic structures forming PyOM.

4.2. Microbial biomass

Microbial biomass and soil respiration decreased over time (Fig. 1d), as it has been shown in many models using microbial

biomass to predict soil respiration (Fang et al., 2005; Fontaine and Barot, 2005). PyOM significantly increased the microbial biomass amount on Days 4, 18 and 88, confirming previous observations by Steiner et al. (2008) and Kolb et al. (2009). The increase may be explained by the easily decomposable fraction initially present in the PyOM (Lehmann et al., 2011) and by the PyOM capacity to host a microbial community (Pietikäinen et al., 2000). Moreover, the increase in soil pH following PyOM addition to the soil may have contributed to increased microbial biomass (Badalucco et al., 1992). The fraction of PyOM incorporated into microbial biomass after 158 days was very low, 0.4% of added PyOM-C, confirming findings by Singh et al. (personal communication), Bruun et al. (2008), and Santos et al. (2012). In contrast, Kuzyakov et al. (2009) observed that, 1.5% of added PyOM, was incorporated into microbial biomass after nearly 20 months incubation. This finding implies that incorporation of PyOM into microbial biomass may be time dependent.

Jones et al. (2012) found that the microbial community of a soil containing PyOM was characterized by a lower microbial efficiency, and hypothesized that this was due to a relative increase in bacteria instead of fungi in the microbial community. In fact, bacteria are known for being characterized by a lower efficiency. This hypothesis was not confirmed in the present study where a significant change in microbial biomass C:N was not observed (Table 1, Supplementary material), a commonly used indicator of the fungal:bacterial composition of microbial biomass (Fierer et al., 2009).

4.3. Temporal mineralization pattern of native soil organic matter

The presence of PyOM promoted the mineralization of native soil organic matter, i.e., induced a positive priming effect, in the first 18 days and inhibited mineralization from Day 18 until the end of the incubation, i.e., induced a negative priming effect (Fig. 1c). Our findings are similar to those by Zimmerman et al. (2011) who hypothesized that in an initial phase, the organic matter promoted the decomposition of PyOM and in a second phase, PyOM sorbed the organic matter and protected it from decomposition. Such a priming effect pattern was also used in the process-based model developed by Woolf and Lehmann (2012) to evaluate the impact of yearly PyOM addition on soil C storage in a maize crop ecosystem over 100 years. In our experiment, the partitioning of soil respiration between PyOM and soil organic matter derived-C indicated that the mineralization of native organic matter was also promoted in the short term, confirming the findings of Keith et al. (2011).

Several processes that may be simultaneous, sequential or mutually exclusive may have occurred to explain these observations. One hypothesis for the initial positive priming effect is that the labile fraction present in PyOM triggers soil microflora. Several authors distinguished two processes occurring in the priming effect that are induced by labile substrates: apparent and real priming effects (Blagodatsky et al., 2010; Kuzyakov, 2010). The apparent priming effect is an increase in the CO_2 efflux resulting from the activation of the dormant biomass due to the addition of available substrates. This results in an increase in the maintenance respiration of the total soil microflora (Blagodatsky and Richter, 1998; Blagodatsky et al., 2010). The real priming effect appears in a second phase and is the result of increased mineralization of the soil organic matter by some of the activated microbes (Kuzyakov, 2010) or by the K-strategist microorganisms. The latter take advantage of the enzymes released by the activated one (Fontaine et al., 2003). The apparent priming effect has often been observed as a result of the addition of labile substrates, e.g., glucose (Wu et al., 1993; Conde et al., 2005; Blagodatsky et al., 2010; Blagodatskaya et al., 2011). Although PyOM is often treated as a homogenous recalcitrant compound it may contain a fraction of easily decomposable substances which have the potential to trigger microbial biomass activity. In our study, the two-pool decomposition model indicated that PyOM also contained a fast pool corresponding to 3.3% of the total PyOM-C (Table 3). The presence of a readily available fraction was confirmed by Hilscher et al. (2009) who observed that ryegrass-derived PyOM contained a fraction of water-soluble C equivalent to 3.9% of PyOM-C. The occurrence of an apparent priming effect in the first four days of the incubation is supported by the following indicators: (i) the easily decomposable fraction of PyOM is lower than the initial microbial biomass (approximately 13% of microbial biomass C) and thus considered to be an insufficient quantity to induce a real priming effect (Blagodatskaya et al., 2011), and (ii) the quantity of primed C after 4 days was lower than the amount of microbial C in the soil (8% of microbial C). This lower level is also assumed to be an indicator of an apparent priming effect (Kuzyakov, 2010).

The positive priming effect may also result from the pH change induced by PyOM addition (Fig. 1 Supplementary materials). Luo et al. (2011) found that the increase in native organic matter mineralization promoted by the presence of PyOM was proportionally higher in acidic than in alkaline soils, suggesting that liming could play a role in determining the magnitude of the positive priming effect. It is well known that liming in acid soils may cause a short-term increase in soil respiration (Badalucco et al., 1992; Haynes and Naidu, 1998; Haynes, 1984). Jones et al. (2011) suggested that PyOM may change the soil pH towards the optimum for extracellular enzymes. In our study, the presence of PyOM increased the pH of soil throughout the entire incubation period (Fig. 1 Supplementary material). However, we observed a change in the direction of priming after 18 days. Therefore, we can only speculate that the change in soil pH was not the prevailing factor responsible for the change in the native soil organic matter mineralization after 18 days. The most often cited explanation for the negative priming effect is that PyOM adsorbs organic matter on its surfaces (Liang et al., 2010; Cross and Sohi, 2011; Zimmerman et al., 2011). Alternatively, the negative priming effect could be explained by depletion in the available substrate (Bingeman et al., 1953). However, this explanation is unlikely in our soil, which had a very high C content, and therefore was not likely to be C-limited. Moreover, Hamer and Marschner (2005) did not observe a limitation in the availability of soil organic C due to the priming effect in a Cambisol incubation in which different substrates were added. We also observed that PyOM caused a decrease in the phenol-oxidase activity (Fig. 3). This could have contributed to a decrease in mineralization of more condensed compounds. However, such decrease was already observed in the first sampling date when the positive priming effect was observed. Moreover, we believe that such a decrease was more likely an artifact of PyOM sorption of the assay (see Section 4.1). Thus, we can only speculate that the reduction in phenol-oxidase may have contributed to the negative priming effect in the second part of the experiment.

4.4. N dynamics

PyOM only altered the NH_4^+ content of the soil up to Day 4 of the incubation period (Fig. 2b and c). NH_4^+ content of PyOM (Table 1) can only explain 26% of the additional NH^{4+} that was recovered on Day 4. Therefore, we concluded that the remaining 74% mineral NH_4^+ was derived from the increased mineralization of the native organic matter (i.e., priming effect) and PyOM mineralization. Moreover, PyOM addition increased gross N mineralization on Day 4 (Kruskal test, $p < 0.05$, Fig. 2c). This confirms the findings of Nelissen et al. (2012) who observed an increase in gross N mineralization in the first week after PyOM addition. By modeling N fluxes using ^{15}N tracer, they found that increased gross N

mineralization was mostly derived from the recalcitrant pool of organic matter. We hypothesize that the increase in gross N mineralization is mainly derived from increased microbial activity, therefore we favor the microflora triggering explanation for priming effect over the pH change one, as liming does affect neither gross (Cheng et al., 2013) nor net (Dancer et al., 1972) N mineralization. Gross N mineralization in the PyOM treatment was also slightly higher than in the control treatment in the fifth sampling date, i.e., after 158 days. The higher N mineralization rate at the end of the incubation period could be due to the mineral N derived from the PyOM decomposition, which in the present study, was shown to have a very low C:N ratio and was therefore a source of N. Moreover, the adsorption of added labeled NH_4^+ onto PyOM surfaces (Jones et al., 2012) may have also reduced the content of labeled NH_4^+ recovered in the extract. This would result in a bias when interpreting gross mineralization data, i.e. the observed NH_4^+ decrease in mineral N pool would be interpreted as an increase of gross mineralization, but low amounts of NH_4^+ were due to its adsorption onto PyOM surfaces and not because of an increase in mineralization per se. The NH_4^+ mineralized within the first few days was rapidly transferred to the NO_3^- pool and remained in this form until the end of the incubation period. Nitrification was very high in our soil. On Day 4, the initial N addition induced a higher nitrification rate, which was probably derived from the nitrification of NH_4^+ from the N added as unlabeled NH_4NO_3 at the start of the experiment. From Day 4 to 18, we found a higher nitrification in the PyOM treatments compared to the control soils likely because of the transformation of the NH_4^+ derived from the strong initial priming effect. Our results disagree with the findings of DeLuca and Sala (2006) who observed higher nitrification rates in burned forest soil compared to unburned. They suggested that PyOM removed nitrification inhibitors, e.g., phenols, derived from shrubs growing in the understory. In the present study, nitrification seemed to be limited by its substrate NH_4^+ rather than by the presence of phenols. Bruun et al. (2012) found that PyOM induced a net N immobilization, while in our study we observed that PyOM induced a net N mineralization. This discrepancy can be explained by the different C:N ratio of the two PyOM (40 and 47, Bruun et al., 2012 versus 9 in the present study). These findings confirm the importance of C:N to predict N mineralization in soils amended with PyOM or other substrates (Mary et al., 1996). The increased N mineralization was not accompanied by an increase in protease activity (Table 2). This is in agreement with the N mining theory that postulates that higher N availability decreases the decomposition of the recalcitrant fraction of a substrate only when it is poor in N (Craine et al., 2007), which was obviously not the case for the PyOM in our study (Table 1). Moreover, the unchanged protease activity in the presence of PyOM might also be because casein is an assay representative of high molecular weight compounds, while the organic matter decomposed at the beginning of the experiment was more likely composed of soluble low-weight N molecules rather than relatively less soluble large molecules.

5. Conclusions

We incubated ryegrass-derived ^{13}C-labeled PyOM for five months in the topsoil of a Cambisol with and without additional N amendments. The PyOM was characterized by a narrow C:N ratio, and mineralized relatively fast. Therefore its efficiency as C-sink in soil system would be rather limited. PyOM promoted native organic matter mineralization during the first 18 days and inhibited it afterward. We suggest that the positive priming effect resulted from an increase in the activity of soil microflora or from the shift in pH following PyOM addition. While negative priming effect may follow from depletion of available C or from the adsorption of organic matter on PyOM surfaces. Our initial hypothesis that N addition may decrease PyOM decomposition via depressing phenol-oxidase activity was not confirmed. On the contrary, PyOM decreased the potential activity of the enzyme, most likely by partly adsorbing the assay. The initial positive priming effect was concurrent to an increase in gross N mineralization and NH_4^+ content. The latter was rapidly nitrified in our soil system. We believe that our results were strongly influenced by the characteristics of the PyOM used, which was characterized by a notably narrow C:N ratio and by the presence of an easily decomposable C-pool. Therefore, we conclude that special attention needs to be paid to PyOM characteristics when evaluating the effect of PyOM on soil C and N dynamics.

Acknowledgments

We are grateful to the anonymous reviewer for providing insightful comments and suggestions that helped to significantly improve the manuscript. We would also like to thank the Swiss National Science Foundation (SNSF) and the University Research Priority Program for Global Change and Biodiversity of the University of Zurich. Moreover, we would like to thank Mirjam Studer for the production of the labeled ryegrass, Michael Hilf, Stefanie Müller, Claudia Schreiner, Julia Siegrist, Nimisha Singh, Annika Tella and Ivan Woodatch (University of Zurich), for help in the laboratory, René Husi (University of Zurich) for the C–N and mineral N analyses, Michael Hösli (Eric Schweizer, Thun) for the mineral N analysis, Matthias Saurer and Ineke Lötscher (Paul Scherrer Institute, Villingen, Switzerland), for the isotopic analysis, and Laura Giagnoni and Loretta Landi (University of Florence) for the enzymatic activity. **Specific contributions:** B. M. developed the experimental setup, performed the analysis, and wrote the article. S. A. contributed to the development of the experimental setup, data analysis, elaboration of the manuscript, and successfully applied for funding the project. A. M. H. contributed to the setup of the ^{15}N pool dilution technique and data analysis, and P. N. contributed to the setup for the measurement of the enzymatic activity. All authors provided input and drafting to the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.11.013

References

Aber, J., Mcdowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., Mcnulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Temperate nitrogen saturation forest ecosystems revisited hypotheses. BioScience 48, 921–934.
Abiven, S., Andreoli, R., 2010. Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. Biol. Fertil. Soils 47, 111–114.
Alef, K., Nannipieri, P. (Eds.), 1995. Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London.
Badalucco, L., Grego, S., Dell'Orco, S., Nannipieri, P., 1992. Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (*Picea abies* L.). Biol. Fertil. Soils 14, 76–83.
Baldrian, P., 2006. Fungal laccases – occurrence and properties. FEMS Microbiol. Rev. 30, 215–242.
Ball, P.N., MacKenzie, M.D., DeLuca, T.H., Holben, W.E., 2010. Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacterial abundance in dry Montane forest soils. J. Environ. Qual. 39, 1243.
Bingeman, C.W., Varner, J.E., Martin, W.P., 1953. The effect of the addition of organic materials on the decomposition of an organic Soil. Soil Sci. Soc. Proc. 17, 34–38.
Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. Soil Biol. Biochem. 43, 778–786.
Blagodatsky, S.A., Richter, O., 1998. Microbial growth in soil and nitrogen turnover: a theoretical model considering the activity state of microorganisms. Soil Biol. Biochem. 30, 1743–1755.

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov, Y., 2010. Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition. Soil Biol. Biochem. 42, 1275–1283.
Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on N cycling rates in soils. Ecol. Monogr. 72, 139–157.
Brodowski, S., Amelung, W., Haumaier, L., Abetz, C., Zech, W., 2005. Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. Geoderma 128, 116–129.
Bruun, S., Jensen, E.S., Jensen, L.S., 2008. Microbial mineralization and assimilation of black carbon: dependency on degree of thermal alteration. Org. Geochem. 39, 839–845.
Bruun, E.W., Ambus, P., Egsgaard, H., Hauggaard-Nielsen, H., 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biol. Biochem. 46, 73–79.
Bruun, S., Clauson-Kaas, S., Bobulská, L., Thomsen, I.K., 2013. Carbon dioxide emissions from biochar in soil: role of clay, microorganisms and carbonates. Eur. J. Soil Sci. http://dx.doi.org/10.1111/ejss.12073.
Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhust, D.F., 2000. Microbial enzyme shift explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.
Chatterjee, S., Santos, F., Abiven, S., Itin, B., Stark, R.E., Bird, J.A., 2012. Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry. Org. Geochem. 51, 35–44.
Cheng, Y., Wang, J., Mary, B., Zhang, J., Cai, Z., Chang, S.X., 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. Soil Biol. Biochem. 57, 848–857.
Conde, E., Cardenas, M., Poncemendoza, A., Lunaguido, M., Cruzmondragon, C., Dendooven, L., 2005. The impacts of inorganic nitrogen application on mineralization of C-labelled maize and glucose, and on priming effect in saline alkaline soil. Soil Biol. Biochem. 37, 681–691.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
Cross, A., Sohi, S.P., 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biol. Biochem. 43, 2127–2134.
Dancer, W.S., Peterson, G.L.A., Chesters, G., 1972. Ammonification and nitrification as influenced by soil pH and previous N Treatments. Soil Sci. Soc. Am. Proc. 37, 67–69.
Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. Annu. Rev. Ecol. Syst. 33, 507–559.
DeLuca, T.H., Sala, A., 2006. Frequent fire alters nitrogen transformations in ponderosa pine stands of the inland northwest. Ecology 87, 2511–2522.
DeLuca, T., Nilsson, M.C., Zackrisson, O., 2002. Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia 133, 206–214.
DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in Ponderosa Pine Forests. Soil Sci. Soc. Am. J. 70, 448.
Egli, P., Maurer, S., Günthardt-Goerg, M., Körner, C., 1998. Effects of elevated CO_2 and soil quality on leaf gas exchange and above-ground growth in beech-spruce model ecosystems. New Phytol. 140, 185–196.
Fang, C., Smith, P., Smith, J.U., Moncrieff, J.B., 2005. Incorporating microorganisms as decomposers into models to simulate soil organic matter decomposition. Geoderma 129, 139–146.
Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.a, Cleveland, C.C., 2009. Global patterns in belowground communities. Ecol. Lett. 12, 1238–1249.
Fontaine, S., Barot, S., 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecol. Lett. 8, 1075–1087.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837–843.
Gaillard, V., Chenu, C., Recous, S., 2003. Carbon mineralisation in soil adjacent to plant residues of contrasting biochemical quality. Soil Biol. Biochem. 35, 93–99.
Goldberg, 1985. Black Carbon in the Environment. Wiley and Sons.
Hamer, U., Marschner, B., 2005. Priming effects in soils after combined and repeated substrate additions. Geoderma 128, 38–51.
Hamer, U., Marschner, B., Brodowski, S., Amelung, W., 2004. Interactive priming of black carbon and glucose mineralisation. Org. Geochem. 35, 823–830.
Hammes, K., Smernik, R.J., Skjemstad, J.O., Herzog, A., Vogt, U.F., Schmidt, M.W.I., 2006. Synthesis and characterisation of laboratory-charred grass straw (Oryza sativa) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. Org. Geochem. 37, 1629–1633.
Haynes, R.J., 1984. In: Brady, N.C. (Ed.), Lime and Phosphate in the Soil–Plant System. Academic Press, pp. 249–315.
Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr. Cycl. Agroecosystems 51, 123–137.
Herrmann, a. M., Witter, E., 2008. Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil. Eur. J. Soil Sci. 59, 653–664.
Herrmann, A.M., Witter, E., Kätterer, T., 2007. Use of acetylene as a nitrification inhibitor to reduce biases in gross N transformation rates in a soil showing rapid disappearance of added ammonium. Soil Biol. Biochem. 39, 2390–2400.
Hilscher, A., Heister, K., Siewert, C., Knicker, H., 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Org. Geochem. 40, 332–342.
Jones, D.L., Murphy, D.V., Khalid, M., Ahmad, W., Edwards-Jones, G., DeLuca, T.H., 2011. Short-term biochar-induced increase in soil CO2 release is both biotically and abiotically mediated. Soil Biol. Biochem. 43, 1723–1731.
Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol. Biochem. 45, 113–124.
Keiluweit, M., Nico, P.S., Johnson, M.G., Kleber, M., 2010. Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environ. Sci. Technol. 44, 1247–1253.
Keith, A., Singh, B., Singh, B.P., 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. Environ. Sci. Technol. 45, 9611–9618.
Khirkham, D., Bartholomew, W.V., 1949. Equations for following nutrient transformations in soil. Soil Sci. Soc. Proc. 1, 33–34.
Knicker, H., 2010. "Black nitrogen" – an important fraction in determining the recalcitrance of charcoal. Org. Geochem. 41, 947–950.
Kolb, S.E., Fermanich, K.J., Dornbush, M.E., 2009. Effect of charcoal quantity on microbial biomass and activity in temperate soils. Soil Sci. Soc. Am. J. 73, 1173.
Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biol. Biochem. 42, 1363–1371.
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biol. Biochem. 41, 210–219.
Lehmann, J., Joseph, S. (Eds.), 2009. Biochar for Environmental Managers. Earthscan, London.
Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biol. Biochem. 43, 1812–1836.
Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Org. Geochem. 41, 206–213.
Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol. Biochem. 43, 2304–2314.
Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Glob. Change Biol. 16, 1366–1379.
Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: theory and models across scales. Soil Biol. Biochem. 41, 1355–1379.
Mary, B., Recous, S., Darwis, D., Robin, D., 1996. Interactions between decomposition of plant residues and nitrogen cycling in soil. Plant Soil 181, 71–82.
Minderman, G., 1968. Addition, decomposition and accumulation of organic matter in forests. J. Ecol. 56, 355–362.
Murphy, D.V., Recous, S., Stockdale, E.A., Fillery, I.R.P., Jensen, L.S., Hatch, D.J., Goulding, K.W.T., 2003. Gross N fluxes in soil: theory measurement and application of ^{15}N pool dilution techniques. Adv. Agron. 79, 69–118.
Nannipieri, P., Eldor, P., 2009. The chemical and functional characterization of soil N and its biotic components. Soil Biol. Biochem. 41, 2357–2369.
Nannipieri, P., Gelsomino, A., Felici, M., 1991. Method to determine guaiacol oxidase activity in soil. Soil Sci. Soc. Am. J. 55, 1347–1352.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci., 655–670.
Nelissen, V., Rütting, T., Huygens, D., Staelens, J., Ruysschaert, G., Boeckx, P., 2012. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biol. Biochem. 55, 20–27.
Novak, J.M., Busscher, W.J., Watts, D.W., Laird, D. a., Ahmedna, M. a., Niandou, M. a. S., 2010. Short-term CO_2 mineralization after additions of biochar and switchgrass to a typic Kandiudult. Geoderma 154, 281–288.
Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231–242.
Ruehr, N.K., Knohl, A., Buchmann, N., 2009. Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry 98, 153–170.
Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biol. Biochem. 51, 115–124.
Schleppi, P., Bucher-Wallin, I., Saurer, M., Jäggi, M., Landolt, W., 2006. Citric acid traps to replace sulphuric acid in the ammonia diffusion of dilute water samples for 15N analysis. Rapid Commun. Mass Spectrom. 20, 629–634.
Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Ko, I., 1999. Charred organic carbon in German chernozemic soils. Eur. J. Soil Sci. 50, 351–365.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
Scott, H.D., Wolf, D.C., Lavy, T.L., 1983. Adsorption and degradation of phenol at low concentrations in soil. J. Environ. Qual. 12, 91.
Singh, N., Abiven, S., Torn, M.S., Schmidt, M.W., 2012. Fire-derived organic carbon turnover in soils on a centennial scale. Biogeoscience 9, 1–11.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.
Grandy, A.S., Sinsabaugh, R.L., Neff, J.C., Stursova, M., Zak, D.R., 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91, 37–49.
Smith, C.J., Chalk, P.M., Crawford, C.M., Wood, J.T., 1992. Estimating gross nitrogen mineralization and immobilization rates in anaerobic and aerobic soil suspensions. Soil Sci. Soc. Am. J. 58, 1652–1660.
Steiner, C., Das, K.C., Garcia, M., Förster, B., Zech, W., 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic ferralsol. Pedobiologia 51, 359–366.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Wang, J., Pan, X., Liu, Y., Zhang, X., Xiong, Z., 2012. Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. Plant Soil 360, 287–298.
Wardle, D.A., Nilsson, M.-C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320, 629.
Wollum, A.G.I., Gomez, J.E., 1987. A conductivity method for measuring microbially evolved carbon dioxide. Ecology 51, 155–156.
Woolf, D., Lehmann, J., 2012. Modelling the long-term response to positive and negative priming of soil organic carbon by black carbon. Biogeochemistry 111, 83–95.
Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biol. Biochem. 25, 1435–1441.
Zavalloni, C., Alberti, G., Biasiol, S., Vedove, G.D., Fornasier, F., Liu, J., Peressotti, A., 2011. Microbial mineralization of biochar and wheat straw mixture in soil: a short-term study. Appl. Soil Ecol. 50, 45–51.
Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J., Zhang, X., 2011. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant Soil 351, 263–275.
Zimmerman, A.R., Gao, B., Ahn, M.-Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 43, 1169–1179.

3 Manuscript III

Priming effect induced by pyrogenic organic matter induced on soil organic matter: a meta-analysis

Bernardo Maestrini, Paolo Nannipieri, Samuel Abiven

The manuscript was accepted for publication in *Global Change Biology-Bioenergy* in March 2014.

Candidate contribution: The candidate conceived the experimental set-up in collaboration with his supervisor Dr. Samuel Abiven. The candidate performed data extraction and analysis. The candidate wrote the manuscript.

A meta-analysis on pyrogenic organic matter induced priming effect.

Bernardo Maestrini[1], Paolo Nannipieri[2], Samuel Abiven[1]

1: University of Zurich, Department of Geography, Winterthurerstrasse 190, Zurich 8057, Switzerland.

2: University of Florence, Department of Plant, Soil and Environmental Sciences, piazzale delle Cascine, 18, 50144 Florence, Italy.

Abstract

Pyrogenic organic matter (PyOM) is considered an important soil carbon (C) sink. However, there are evidences that its addition to soil may induce a priming effect (PE) thus influencing its C abatement potential. The direction, the size and the mechanisms responsible for PyOM induced PE are far from being understood. We collected approximately 650 data points from 18 studies to analyze the characteristics of the PE induced by PyOM. The database was divided between the PE induced on the native soil organic matter and on fresh organic matter. Most of the studies were short-term incubation therefore the projections of findings on the long term may be critical. Our findings indicate that over one year PyOM induces an average positive PE of 0.3 mg C g-1 soil on native soil organic matter and a PE of approximately the same size but opposite direction on fresh organic matter. We studied the correlation of PE with several properties of soil, of the added PyOM, and time after PyOM addition. We found that PyOM primes positively the native soil organic matter in the first 20 days while negative PE appears in a later stage. Negative PE was correlated to the soil C content. PyOM characterized by a low C content induced a higher positive PE on native soil organic carbon.

No correlation was found between the factors record in our database and the PE induced on the fresh organic matter.

We reviewed the mechanisms proposed in literature to explain PE and discussed them based on findings from our meta-analysis. We believe that the presence of a labile fraction in PyOM may trigger the activity of soil microorganisms on the short term and therefore induce a positive PE, while on the long term PyOM may induce a negative PE by promoting physical protection mechanisms.

Introduction:

Pyrolysis process consists in the heating of organic matter under anoxic conditions. Pyrolysis can occur during wildfires, where local and temporary limitation of oxygen can occur, or it can be a controlled process to produce heat and PyOM, also termed *biochar,* from agricultural residues (Lehmann & Joseph, 2009). We define here Pyrogenic organic matter (PyOM) as the residues of pyrolysis of biomass. PyOM can represent an important fraction of soil C both in soils amended with biochar and in natural soils. In fact PyOM is virtually present in every soil (Preston & Schmidt, 2006), and massive inputs of PyOM to the soil occur every year by forest burning. The estimations of PyOM global production vary between 40 to 600 Tg C $year^{-1}$ (Crutzen & Andreae, 1990; Kuhlbusch & Crutzen, 1995). Such a high input rate together with its centennial mean residence time (Singh *et al.*, 2012a) makes PyOM a fundamental component of soil organic matter. Moreover, PyOM content in soil is likely to increase in future due to the increasing fire frequency (Moritz *et al.*, 2012; Flannigan *et al.*, 2013) and to the growing interest in the use of PyOM - biochar - as a tool to increase soil fertility and store C at the same time (Lehmann, 2007; Verheijen *et al.*, 2010).

Many recent studies indicated that PyOM beside from being a stable C pool can also affect the stability of non-PyOM C pools, i.e. it may induce a priming effect (PE). Here we adopt the terminology from Bingeman *et al.*, (1953) who use the term PE to indicate the change in the mineralization rate of the soil organic matter in a soil receiving an input of exogenous organic matter. We specifically define PE as positive when the addition of PyOM increases the mineralization rate of non-PyOM C and negative when PyOM decreases the mineralization rate of non-PyOM C. PyOM was previously found to induce a positive PE (Wardle *et al.*, 2008; Luo *et al.*, 2011) or a negative PE (Kuzyakov *et al.*, 2009; Cross & Sohi, 2011). Also, no effect has been reported (Abiven & Andreoli, 2010; Santos *et al.*, 2012).

In the literature, PE was often reported as a response of soil to the addition of easily decomposable substrates like sugar (Wu *et al.*, 1993; Conde *et al.*, 2005; Blagodatskaya *et al.*, 2007, 2011; Blagodatsky *et al.*, 2010; Kuzyakov, 2010), cellulose (Fontaine & Barot, 2005) or plant material, e.g. rye grass (Wu *et al.*, 1993). Surprisingly it was found that also PyOM induced a priming effect although it has a low microbial availability (Kuzyakov *et al.*, 2009; Singh *et al.*, 2013). Several mechanisms have been proposed to explain the PE observed in individual studies; however a comprehensive analysis of results and mechanisms is still lacking.

Predicting the importance of PE induced by PyOM on the mineralization of non-PyOM C is crucial to assessment of PyOM C abatement potential. Woolf & Lehmann (2012), predicted the importance of PyOM induced PE on C stabilization in agroecosystem by using a process based model, however very little is known on the mechanisms responsible for PyOM induced PE. For example, it is still not known whether the PE is related to the type of soil and to the characteristics of the primed soil organic matter, or to the characteristics of the PyOM. It is also not known whether the PE is important on the long term, or if it is only a short-term phenomenon. The aim of the present study is to assess whether PyOM induces PE, its impact

on soil C budget, and which factors are driving its size and direction. To reach these goals we carried out a meta-analysis of the data reported in literature on PyOM induced PE separately on native soil organic matter and on fresh organic matter. We define here fresh organic matter, as an organic input added to the soil, e.g. plant material or glucose.

Moreover we reviewed the existing mechanisms presented in literature and discussed them based also on results from our meta-analysis.

Data collection

The PE induced by PyOM on the native organic matter and on the fresh organic matter was considered separately. We extracted data from individual sampling dates, in order to take into account the effect of time on PE. We expressed PE as the increase in CO_2 efflux derived from non-PyOM C pool compared to CO_2 efflux in the control treatment, i.e. in the treatment without PyOM addition. Where CO_2 efflux was not expressed as rate but as cumulative CO_2 efflux, we transformed the cumulative values into fluxes and attributed it to the last day of the cumulative period. To give the same importance to each study, we attributed to each data point a weight, inversely related to the number of data extracted from the study, using equation [1]:

$$W=1/N_{data} \quad [1]$$

Where W is the weight attributed to individual data, and N_{data} is the number of data extracted from the study.

To estimate how soil respiration changed over time in treatments where no PyOM was added (i.e. in control treatments) we used a linear regression of soil respiration on log-transformed time (Figure 1 supplementary material). For PE on native soil organic matter we restricted our data collection to studies employing isotopic techniques to discriminate among the sources of

94 CO_2. In the case of studies using both labelled and unlabelled techniques (Zimmerman *et al.*,
95 2011) we considered only the treatments with ^{13}C-CO_2 estimations. We consider here that
96 only the ^{13}C-based studies can provide reliable information and direct calculation to the PE.
97 For the PE on native organic matter, we collected data from 12 publications (Table 1). Our
98 data set was composed in total of 464 data points, each of them reporting PyOM C content,
99 soil C content, time, soil pH, PyOM-C added g^{-1} soil C added, PyOM parent biomass (wood
100 or grass), soil texture (classes).

101 PE was calculated using equation 2:

102 $$PE = NSOM_{PyOM} - NSOM_{con} \quad [2]$$

103 Where PE = PE, $NSOM_{PyOM}$ = mineralization rate of Native Soil Organic Matter in soil
104 amended with PyOM, $NSOM_{con}$ = mineralization rate of Native Soil Organic Matter in control
105 (soil without any amendment). All the terms are expressed as mg $C\text{-}CO_2$ g^{-1} soil day^{-1}.

106 For the PE induced by the interaction between PyOM and fresh organic matter, we compiled a
107 data set from six studies (Table 2), consisting in a total of 198 data. We estimated PE using
108 equation 3:

109 $$PE = (MIN_{withPyOM} - MIN_{withoutPyOM}) / MIN_{withoutPyOM} \quad [3]$$

110 Where $MIN_{withPyOM}$ is the mineralization of the fresh organic matter in the PyOM treatment
111 and $MIN_{withoutPyOM}$ the mineralization in the control treatment.

112 In the studies where PyOM induced PE on fresh organic was measured in a soil matrix (i.e.
113 not in quartz sand like in Hamer *et al.*, (2004), it is necessary to partition the CO_2 between
114 three sources: PyOM, native soil organic matter, and fresh organic matter. This would imply
115 the use of two of different isotopic labels, i.e. ^{14}C and ^{13}C, as in the study from Blagodatskaya
116 *et al.*, (2011). Since such set up has not been used so far in studies on fluxes in PyOM
117 amended soils, we included also studies where isotopic techniques were not applied.

Therefore when it was not possible to separate the flux of fresh organic matter from the other flux we used equation 4:

$$PE = (SR_{observed}-SR_{theoretical})/SR_{theoretical} \quad [4]$$

Where $SR_{observed}$ was the SR in $SR_{(soil+fresh\_organic\_matter+PyOM)} - SR_{soil}$, and $SR_{theoretical}$ was the sum of $(SR_{fresh\_organic\_,matter+soil}-SR_{soil})+(SR_{PyOM+soil}-SR_{soil})$, therefore attributing the whole change in SR to the a change in the mineralization of the fresh substrate. We reported the PE as % of fresh organic matter decomposition rate. We also calculated the PE and expressed it as mg C g C-fresh organic matter added, using equation 5 and 6:

$$PE= (MIN_{withPyOM} - MIN_{withoutPyOM}) \quad [5]$$

$$PE = (SR_{observed}-SR_{theoretical}) \quad [6]$$

To compare PE on fresh organic matter with the PE on native soil organic matter, we then expressed it as mg $C\text{-}CO_2$ g^{-1} soil day^{-1}. We recognise, however that this value is affected by the quantity of fresh organic matter added to the soil in the different experiments.

We tested the following factors as explanatory variables for PE induced on fresh organic matter: time, fresh organic matter C:N ratio, PyOM-C, PyOM-N, PyOM-C:N ratio, fresh organic matter N content, fresh organic matter C, fresh organic matter addition rate and soil C.

To evaluate which factors are influencing PE, we used the model simplification approach described in Crawley (2007) consisting in seeking for the minimal adequate model. In order to estimate if the relation between the response variable (PE) and the explanatory variable was significant without presuming their distribution, we used the bootstrapping method with 1000 re-sampling iterations (Crawley, 2007) to establish whether the slope of the regression was significantly different from 0 ($p<0.05$). The regression was weighted by giving to each point

the weight in equation 1. All the statistical analyses were performed using the statistical software *"R"*. The software *"g3Data" (*http://www.frantz.fi/software/g3data.php*)* was used to extract data from the figures.

Results and discussion of the meta-analysis

The maximum positive PE on native soil organic matter mineralization was observed by Cross & Sohi, (2011) in an incubation experiment fifteen days after PyOM addition and was equal to 0.04 mg C-CO_2 g^{-1} soil day^{-1}, while the minimum PE (i.e. the maximum negative PE) observed was equal to -0.02 mg C-CO_2 g^{-1} soil day^{-1} after 90 days (Zimmerman *et al.*, 2011). The maximum observation period was the incubation experiment by (Singh *et al.*, 2012b), lasting for 5 years. Overall most of the data points collected in the data base were short-term measurements. In fact 50% of the data points we collected were measured within less than three months after PyOM addition to the soil (Figure 2 supplementary material). Overall the average weighted PE observed in literature was 0.0020±0.0003 mg C-CO_2 g soil day^{-1} ($p<0.001$, weighted t-test). Among the explanatory variables, time (logarithm transformed) and the interaction between time and PyOM-C content were significant ($p<0.001$, regression slope different from 0, boot strapping). We observed that PE decreased with time, and that PyOM having a low C content induced more positive PE on the short term (Figure 3 supplementary material). Most of the highest positive effect occurred within the first 20 days and with a low PyOM-C content, while most of the negative priming occurred on a longer time scale (Figure 2). Modelling PE over time, based on data collected in the database, we observed that the PE was positive until 200 days, and then negative (Figure 1). When PE was cumulated over time it was reaching a neutral PE, i.e the positive PE induced in the beginning was counterbalanced by the negative observed afterward, approx 600 days (Figure 1). We did not observe a correlation between PE and the other variables recorded in our database: PyOM

C content, soil C content, time, soil pH, PyOM-C added g^{-1} C added, PyOM parent biomass (wood or grass), soil texture (classes).

We calculated the integral of the curve relating PE and time, and we found that PyOM after one year of addition induced a cumulative positive PE equivalent to 0.3 mg C g^{-1} soil. This loss represented 15% (0.32 mg C–CO_2 g^{-1} $soil^{-1}$, Figure 1) of the average soil respiration in control treatment, i.e. where no PyOM was added (2.1 mg C–CO_2 g^{-1} $soil^{-1}$, Figure 1 supplementary). The theoretical influence of PE on the abatement potential of PyOM-C is reported in Figure 4 of the supplementary materials. Given that we did not find a correlation between PE and the amount of PyOM-C added to the soil, we considered a fixed PE of 0.3 mg C g^{-1}soil $year^{-1}$. The impact of PE on C abatement potential is inversely proportional to the amount of PyOM-C added g^{-1} soil. Therefore when low amounts of PyOM are added to the soil the impact of PE can be relevant. In the studies collected in our database the mode of the addition rate was about 10 mg PyOM-C g^{-1} soil (Figure 4 supplementary), in this case losses by PE would represent 3% of PyOM-C added. Such addition rate would correspond in an ideal soil having 1 g cm^{-3} and tilled down to 20 cm to an addition rate of 20 t PyOM-C $ha^{-1.}$ Considering a pyrolysis C yield of 37% (Woolf & Lehmann, 2012) approximately 54t of agricultural residues-C would be necessary to produce such amount of PyOM. This would correspond to the summation of the agricultural residues produced over several years. Therefore we believe that the quantity of PyOM added to the field would generally be lower than the mode of PyOM generally added in laboratory experiment and therefore PE may significantly reduce the abatement potential of PyOM.

In the data set on PE induced on fresh organic matter the longest observation period was 1.5 years in the incubation experiment by Liang *et al.*, (2010). The maximum observed PE was 53 % and was observed in Novak *et al.*, (2010) after 25 days, while the minimum was observed in Zavalloni *et al.*, (2011) where the decomposition of fresh organic matter plus PyOM did not

significantly differ from the decomposition of fresh organic matter alone. We found that the mean of PyOM induced PE on the fresh organic matter was negative and was -10±2% (weighted t-test, $p<0.001$) of the theoretical mineralization rate, this is equivalent to -3.9±1.5 mg C-CO_2 g C-fresh organic matter day^{-1} and to -0.005±0.002 mg C-CO_2 g^{-1} soil day^{-1} when expressed on a g of soil basis. We could not find a relation between recorded factors and PE. Nevertheless this could be attributed to the setups that differed considerably among the experiments, and this may have increased the variability of the response.

Woolf & Lehmann (2012) predicted using a process based model, that the cumulative PE induced by PyOM, at the end of the year, was negative. They assumed that the C stabilization effect of PyOM was linearly related to amount of PyOM content in soil, while we observed a correlation between PyOM-C and PE and no correlation with the application rate. If negative PE is considered to be positively correlated to PyOM content in soil, assuming a yearly input of PyOM to the soil, and given the low decomposition rate of PyOM, the C stabilization will increase year after year. Given our results we believe that assuming the C stabilization to be proportional to PyOM contained in soil, may lead to an overestimation of C stabilized particularly when predicting dynamics over decades. Moreover Woolf & Lehmann (2012) considered only the effect of PyOM on the fresh organic matter, for which however very few experiment exist (Table 2), and did not take into account the effect that PyOM can have on native soil organic matter which may counterbalance the impact on the fresh organic matter, as we observe from our results that the two effects have an opposite direction.

When looking at the PE induced by other types of organic matter, Sayer *et al.* (2011) found that litter input increased native soil organic mineralization by 13% over one year, while Crow *et al.* (2009) found that litter addition induced a priming of 15-21% over one year. This indicates that the PE induced by PyOM is equivalent to other types of organic inputs, despite its low decomposability.

Based on this meta-analysis, we conclude that both negative and positive PEs can co-exist. Looking at the native soil organic matter, the positive PE occurs on the short term and more intensively, while negative PE is acting on the longer term with less intensity. The characteristics of the PyOM (C content) also seem to play a role, but this is directly related to the time, i.e. PyOM characterised by a low C content may induce a positive PE on the short term. While for the PE on the fresh organic matter it was not possible to establish a relationship between the observed PE and the variables recorded in our database. Also, it has to be noticed that most of the studies included in the present meta-analysis are short-term (Figure 2 supplementary) and therefore projections of the impact of PE on the long term, may suffer from a lack of data. Moreover it has to be considered that on the long term the factors that may influence PE size and direction may vary, e.g. repeated addition of PyOM, seasonal variations of soil temperature and moisture. As these factors were constant in most of the studies considered (incubation studies) it was not possible to consider the effect of their variation over time.

In the literature, several mechanisms have been proposed to explain the PE induced by PyOM. In the following sections, we will review these mechanisms and evaluate how influential they can be considering also the meta-analysis results. We believe that the mechanisms hereafter described do not exclude each other and they may all contribute simultaneously to the resulting net PE.

The labile fraction mechanism

Our findings on the positive PE induced on native soil organic matter agree with the theory of Fontaine *et al.* (2003) and suggest that the addition of fresh organic carbon to the soil represents an energy source that increases the microbial biomass. According to the theory from Fontaine *et al.* (2003) if the added substrate is sufficiently complex this will favour the

239 growth of k-strategist decomposers responsible for the decomposition of the native organic
240 matter. In fact several studies observed an increase of soil microbial biomass after PyOM
241 addition (Bruun *et al.*, 2008; Steiner *et al.*, 2008a; Kolb *et al.*, 2009; Lehmann *et al.*, 2011;
242 Maestrini *et al.*, 2014a). Moreover PyOM, being a complex blend of molecules (Schmidt &
243 Noack, 2000; Keiluweit *et al.*, 2010; Spokas, 2010), is likely to represent the kind of substrate
244 that can trigger the growth of k-strategists microbes.

245 In addition, PyOM may contain a small easily decomposable fraction (Hamer *et al.*, 2004;
246 Keith *et al.*, 2011; Santos *et al.*, 2012; Singh *et al.*, 2012b; Maestrini *et al.*, 2014a) that would
247 constitute an energy source for microbial community on the short term. This agrees with our
248 findings that low-C content PyOM induce positive priming on the short term (Figure 1
249 supplementary materials). Several studies show that PyOM characterised by a low C content
250 contains a larger labile fraction. Singh *et al.*, (2012b), using ^{13}C cross polarization NMR
251 found that a low C content in PyOM was associated with a larger fraction of non aromatic C.
252 Fabbri *et al.*, (2012) found that a higher PyOM content in sugar, estimated by pyrolysis
253 coupled to gas-chromatography, was often associated to a low C content. Singh *et al.*, (2012b)
254 also found a positive correlation between soil respiration and the presence of an easily
255 decomposable fraction and Fabbri *et al.*, (2012) found that higher CO_2 fluxes were associated
256 to PyOM containing a higher fraction of sugars and a low level of aromaticity.

257 Even though we did not find a correlation between PyOM C content and pyrolysis
258 temperature there is a general agreement in the literature on the positive correlation between
259 the two (Keiluweit *et al.*, 2010; Zimmerman *et al.*, 2011). Also, the pyrolysis temperature is
260 likely to affect PyOM chemistry and thus its availability to soil microorganisms. Keiluweit *et*
261 *al.*, (2010) proposed a multi-phase model that correlates PyOM structure and pyrolysis
262 temperature. Their model proposes that the PyOM content of volatile matter (which is thought
263 to be more labile) decreases with higher pyrolysis temperature while the non-volatile fraction

(more resistant to decomposition) shows a relative increase. In this model a distinction is made between amorphous PyOM and composite PyOM. The amorphous PyOM is composed mainly of pyranones, phenols, pyrroles and is characterised by a relative increase of stable aromatic lignin compared to raw material, since lignin is more heat resistant than cellulose. The turbostratic PyOM is formed at higher temperature and is characterised by a higher degree of condensation. Turbostratic PyOM is composed of turbostratic crystallites embedded in a matrix of amorphous PyOM. It is therefore possible that less condensed and thus more available PyOM produced at low temperatures stimulates soil microorganisms inducing a higher positive priming effect.

Also the feedstock may affect PyOM chemical properties like PyOM-C content and forms. It is known that grass-derived PyOM are characterized by a lower C content and thus are less condensed, probably due to the lower thermal stability of cellulose compared to lignin (Hammes *et al.*, 2006; Keiluweit *et al.*, 2010; Knicker, 2010). For example, Hilscher *et al.*, (2009) using ^{13}C NMR technique, observed that a low C content of the ryegrass-derived PyOM corresponded to a higher content of alkyl C compared to the wood-derived PyOM. Overall we can therefore expect that grass-derived and low temperature PyOM induce a higher positive PE on the short term.

Sorption

This mechanism is related to the capacity of PyOM to adsorb other organic compounds. This property is already described extensively in the literature (Lehmann *et al.*, 2005; Lehmann & Joseph, 2009; Joseph *et al.*, 2010). PyOM capacity to adsorb organic compounds is related to its high porosity and cation exchange (Lehmann, 2007). It has been shown that PyOM has a higher sorption capacity than non-PyOM for dissolved organic matter (Cornelissen *et al.*, 2005a) and organic xenobiotics (Cornelissen *et al.*, 2005b).

Carbon stabilization by sorption is generally considered one of the most likely mechanisms for PyOM induced negative PE (Kimetu *et al.*, 2009; Kuzyakov *et al.*, 2009; Cross & Sohi, 2011), even though there is very little evidence for sorption of dissolved organic carbon to depress organic matter decomposition (Kaiser & Guggenberger, 2000). The PyOM sorption properties have been proposed as an explanation for negative PE from different perspectives. First, the PyOM has been hypothesized to adsorb part of the native soil organic matter, leading to a lower availability of substrate for decomposers (Kimetu *et al.*, 2009; Kuzyakov *et al.*, 2009; Cross & Sohi, 2011). Pietikäinen *et al.*, (2000) observed a high capacity of PyOM to adsorb dissolved organic carbon compared to pumice. Second, it has been proposed that PyOM sorption potential may influence enzyme activity. Zimmerman *et al.*, (2011) suggested that PyOM stabilization of extracellular enzymes alters their activity by: (i) blocking the active sites of the enzyme or (ii) by inducing a deformation on the tertiary structure of the enzyme. However these hypotheses were not confirmed by Jin, (2010) and Bailey *et al.*, (2011) who found an inconsistent effect of PyOM on a wide range of enzyme activities: the addition of PyOM to the soil in some cases increased enzyme activity, while decreased it in others. They attributed the increase in the activity to the PyOM stimulation of soil microorganisms and the decrease to the sorption of the assay on PyOM surface. Moreover, it has to be considered that among the two forms of enzymes active in the soil, the extracellular stabilized enzymes and the intracellular enzymes, only the latter contribute directly to microbial activity (Nannipieri & Gianfreda, 1998; Nannipieri *et al.*, 2012). The ecological role of extracellular stabilized enzymes is only indirectly connected to microbial activity as they may serve as a reservoir of potential enzymatic activity in case of changes in substrate availability and as originator of signalling molecules by cleaving off small fragments of larger polymer. The signalling molecules act as an inducer for microbes to release the enzyme for the target molecules (Gianfreda & Rao, 2011; Wallenstein & Burns, 2011). Therefore we believe that the sorption of extracellular enzymes on clays and organic matter (including

314 PyOM) can not decrease the microbial activity, although it can decrease the enzyme activity
315 measured using the classical approach which can not separate between extracellular enzymes
316 and enzymes associated to microbes (Nannipieri *et al.*, 2012).

317 In our meta-analysis the size of the negative PE was higher when the soil had a higher content
318 in C when data on negative PE are pooled together (Figure 3). The sorption of dissolved
319 organic carbon agrees with these findings: in fact the dissolved organic carbon production is
320 not directly related to the time, and the physical protection mechanism is likely to become
321 relatively more influential in a second stage when the positive PE has ceased. Also, the
322 dissolved organic carbon production is proportional to the amount of C in the soil (Kalbitz *et*
323 *al.*, 2000). This confirms findings from Zimmerman *et al.*, (2011), and Stewart *et al.*, (2013)
324 who observed a higher C stabilization in soil richer in PyOM over a two years incubation.
325 Zimmerman *et al.*, (2011) suggests that soil more rich in C will produce more C stabilization
326 due to the higher potential C to be sorbed. However, Kuzyakov *et al.*, (2009) observed C
327 stabilization in a loess sediment (very poor in C) but not in a C richer loess derived-soil,
328 which although it was generally poor in C was still ten times richer in C than the loess
329 sediment. Also, Chen *et al.*, (2008) showed that while non-PyOM sorption capacity is linearly
330 dependent on the concentration of the solute the PyOM sorption capacity is non-linearly
331 dependent, and shows greater affinity at low concentration rates, challenging the theory that
332 negative PE is correlated with DOC content of soil. Nevertheless, the range of concentrations
333 where PyOM sorption capacity is not linear is lower than the typical concentration of
334 dissolved organic carbon in soil (mg C L^{-1}), therefore in the soil the sorption capacity of
335 PyOM is likely to be linearly correlated in the concentration range of dissolved organic
336 carbon.

337 Chun *et al.*, (2004) and Chen *et al.*, (2008) found that the pyrolysis temperature significantly
338 altered the sorptive capacity of PyOM. They found that pyrolysis temperature altered the ratio

339 of carbonized/non-carbonized material that are characterised by different adsorption
340 properties. Also James *et al.*, (2005), Bornemann *et al.*, (2007) and Harvey *et al.*, (2011)
341 found similar results with increasing sorption affinity positively correlated to pyrolysis
342 temperature. It is therefore possible that charcoal produced at higher temperature has higher
343 sorption capacity. This would strengthen the importance of PyOM-C content which maybe
344 correlated on the one side to the size of the PyOM labile C content and on the other for PyOM
345 sorption capacity. Therefore high temperature PyOM may contribute to stabilize C in the soil,
346 while low temperature C may contribute to stimulate microbial activity.

347 Overall the sorption of dissolved organic matter on PyOM surface is a likely explanation for
348 the negative PE, particularly it is possible that in concurrence with the labile fraction it
349 determines the temporal pattern of PE of initial positive PE followed by a phase of negative
350 PE, as observed in several studies (Singh *et al.*, 2012b; Maestrini *et al.*, 2014a).

351 **Mechanisms related to the changes of soil properties**

352 Addition of PyOM to the soil may change the soil chemical and physical properties. There is
353 an increasing body of evidence that PyOM addition will increase soil pH, mostly by adding
354 ash to the soil (Van Zwieten *et al.*, 2009; Liu & Zhang, 2012; Maestrini *et al.*, 2014a). Luo et
355 al., (2011) observed a relatively higher initial PE in a soil with a lower pH, however we did
356 not find a relation between priming and soil or PyOM pH (data not reported). Nevertheless
357 the alteration of pH may affect organic matter mineralization in several ways. When the pH of
358 soil solution is higher than 5 this may alter the equilibrium between CO_2 and carbonates
359 (HCO_3^-, and CO_3^-) promoting the formation of the latter. This results in an artificial lowering
360 of the CO_2 efflux until the saturation of the soil solution is reached, this generally occurs
361 within a few days (Blagodatskaya & Kuzyakov, 2008; Lehmann *et al.*, 2011). Theoretically
362 the relevance of this process decreases with time, as PyOM amendment effect on pH has been

363 shown to decrease (Cheng *et al.*, 2006; Maestrini *et al.*, 2014a). However we observed that
364 most of the negative PE occurred in later stages, therefore we believe that this is not a main
365 driver for observed negative PE.

366 A shift in soil pH by PyOM addition may affect enzymatic activity (Jones *et al.*, 2011). Even
367 though we cannot rule out this hypothesis, each enzyme has its optimum and it is therefore
368 likely that a shift in pH does not have a unidirectional effect on enzyme activity.

369 Kimetu & Lehmann, (2010) suggested that PyOM may increase soil aggregation which can in
370 turn increase C stabilization, particularly by incorporating C in microaggregates (Six *et al.*,
371 2006). However, it is often not possible to distinguish the effect of sorption from the effect of
372 aggregation, in fact these two effects are often classified together as physical protection
373 mechanisms (Sollins *et al.*, 1996). Vasilyeva *et al.*, (2011), Brodowski *et al.*, (2006) and
374 Skjemstad *et al.*, (1993), found relatively large quantities of PyOM in microaggregates, and
375 propose that PyOM plays an important role in microaggregates formation acting as a binding
376 agent. A similar conclusion was drawn by Fellet *et al.*, (2011) who observed a higher water
377 retention capacity in PyOM amended soil and attributes this to an increase of aggregation.
378 Piccolo *et al.*, (1997), found that coal derived humic substances can improve aggregate
379 stability by creating a water repellent coating to the humic-mineral associations. Liang *et al.*,
380 (2010) found that PyOM increased the incorporation of fresh added organic matter in the
381 aggregates. However in their study this was not accompanied by a decrease of the PE induced
382 by PyOM on the fresh organic matter. We would expect that the promotion of aggregates
383 formation would have pronounced effects in soil with little structure, like sandy soil, however
384 we did not find an effect of soil texture on PE (data not reported), and therefore although we
385 believe that this mechanism can occur we can not confirm this with our meta-analysis.

386 An alternative explanation for the phase of negative PE observed in several studies is the
387 shortage of available organic matter caused by the higher decomposition rates of the positive

388 PE phase (Bingeman *et al.*, 1953). However, Hamer & Marschner, (2005) have shown by
389 repeated substrate addition that there was no limitation in availability of soil organic carbon to
390 PE. Moreover when this mechanism occurs we expect that a soil richer in C would be less
391 affected by C depletion effect, but results on negative PE did show that soils richer in C are
392 affected by more negative PE.

393 Mechanisms related to the change in microbial community and 394 activity

395 The shift in the microbial structure is an explanation that has been used to explain both
396 positive and negative PE. Zimmerman *et al.*, (2011) proposes that compounds toxic to
397 microbes can be released from the PyOM and reduce microbial activity and organic matter
398 mineralization. However they reject this hypothesis since the toxicity should show its effect
399 on microbial biomass on the short term, due to the volatile fraction they contain. Our results
400 on the PE occurring mostly on the short term, confirm that even if such mechanism exists it is
401 not prevalent at least on the short term. Moreover there is also an increasing body of evidence
402 reporting an increase in microbial biomass following PyOM addition that indirectly do not
403 confirm the presence of toxic compounds (Bruun *et al.*, 2008; Steiner *et al.*, 2008b; Kolb *et*
404 *al.*, 2009; Schneider *et al.*, 2011; Maestrini *et al.*, 2014a). Nevertheless, it is also possible that
405 the observed increase in microbial biomass derives from an increased turnover following from
406 the death of microbial groups sensitive to the toxic compounds. In this case the dead
407 microbial cells could represent a growing substrate for microbes less sensitive to the toxic
408 elements of PyOM, generating an increased turnover of microbial biomass (Blagodatsky *et*
409 *al.*, 2010).

410 Lehmann *et al.*, (2011) proposed based on the observation that PyOM induces a negative PE
411 and an increase in microbial biomass that the PyOM capacity of adsorbing organic matter

412 increases microbial efficiency (i.e. microbes produce less CO_2 for the same amount of C
413 incorporated). They suggested that microbial biomass increase may be due to co-location of
414 micro-organisms and soil organic matter; this would reduce the energy invested by microbes
415 in producing extracellular enzymes. The basis for this hypothesis is the *autoinducer* theory
416 (Redfield, 2002) that postulates that microbes release the enzymes only when the risk of
417 losing them by diffusion is minimal. According to such theory a concentration of
418 decomposable substrate higher than the quorum sensing, i.e. the substrate concentration
419 threshold for bacteria to produce extracellular enzymes, will induce the production of
420 extracellular enzymes, and therefore increase substrate decomposition and minimize the
421 expenses for the production of enzymes.

422 Our database does not allow us to verify the hypotheses on the efficiency of microbial
423 biomass in PyOM amended soils, however our results on positive PE induced by PyOM on
424 the short term are challenging the initial assumption from Lehmann *et al.*, (2011). Also Jones
425 *et al.*, (2012), who estimated microbial efficiency based on initial consumption rate of added
426 glucose found that microbial efficiency in PyOM amended soil decreased, therefore not
427 confirming this hypotheses.

428 Wardle *et al.*, (2008) observed in a litter bag experiment an increase in C mineralization from
429 a mix of charcoal and humus compared to the expected decomposition from the two
430 substrates separately. They suggested that PyOM can act as a *"foci"* (Zackrisson *et al.*, 1996),
431 i.e. a spot, where microbes can grow and decompose the phenols sorbed on PyOM surface
432 being protected from microarthropod predation. In fact according to Warnock *et al.*, (2007)
433 and Zackrisson *et al.*, (1996) the size of many charcoal pores (often below 16 µm in
434 diameters) would allow the entrance of bacteria, fungi and microbe-feeding nematodes but not
435 of predators like protists (8-100 µm) nor micro-arthropods (100µm-2mm). However, as
436 Lehmann *et al.*, (2011) recently reviewed there is no quantitative evidence that microbes

437 inside the PyOM porosity are protected from predators. Moreover the theory that microbial
438 activity is enhanced by protection against predation is challenged by the many evidences that
439 predation of microbes increase CO_2 efflux for example by increasing the activity of soil
440 microorganisms by transporting them to unexploited substrates and by providing excretion
441 and defecations that are readily usable (Ingham *et al.*, 1985).

442 Mechanisms for priming effect on fresh organic matter

443 The mechanisms proposed in literature for PE on fresh organic matter do not differ
444 substantially from the one proposed for the native soil organic matter. Wardle *et al.*, (2008)
445 suggests the foci hypotheses, similarly Hamer *et al.*, (2004) proposed that PyOM offers a
446 large surface for the growth of microorganisms. Zavalloni *et al.*, (2011) observed a
447 stabilization effect mixing PyOM and wheat straw, and suggested that the addition of PyOM
448 and decomposable organic matter produces an increase of C immobilization in microbial
449 biomass. However incubations are generally short term, and therefore the possible
450 immobilization of C in microbial biomass would be released on the long term by microbial
451 turnover. Jones *et al.*, (2012) suggests that PyOM induces a shift in the microbial community
452 promoting less C-efficient microorganisms, so that more C is respired and less is stored. Keith
453 *et al.*, (2011) report that PyOM may stabilize fresh organic matter by trapping it into organo-
454 mineral fractions. This was confirmed by Liang *et al.*, (2010) who found that more fresh
455 organic matter was incorporated in the aggregate fraction in a soil containing high quantities
456 of aged PyOM.

457 Looking at all the different reported explanation it appears clear that the labile theory does not
458 apply for the fresh organic matter, in agreement with the principle that fresh organic matter is
459 generally a source of energy for microbes, while old soil organic matter is rather a source of
460 nutrients (Fontaine *et al.*, 2003). On the other hand soil organic matter is rich in soluble
461 compounds that once in soil can be mobilised and trapped in the aggregates whose formation

462 is promoted by the presence of PyOM. Although the limited number of studies and the
463 heterogeneity of their setup does not allow us to conclude on the definitive mechanisms
464 responsible for the often reported PE on the fresh organic matter, we believe that it is likely
465 that PyOM may stabilize fresh organic matter by physical protection mechanisms.

466 Research perspectives

467 Although much work has been done on the mechanisms responsible for the priming effect, a
468 big uncertainty still resides in the processes occurring at landscape levels that may influence
469 PE induced by PyOM. We believe that particularly three aspects has to be elucidated: (i)
470 impact of continuous input of organic matter, (ii) impact of different land uses. Very little is
471 known on the impact of continuous input of fresh organic matter, their impact can be partially
472 inferred from studies where fresh organic was repeatedly added to the soil and biochar mix
473 (Hamer et al., 2004, Kuzyakov et al., 2009). Nonetheless, repeated input of the same
474 substance or mix of substances can hardly reproduce of the diversity and continuity of
475 rhyzodeposition.

476 Land use is a relevant driver for organic matter dynamics in soils, for example compaction, or
477 litter production seasonality strongly influence decomposition. Nonetheless so far studies
478 investigating PyOM induced PE only looked at the influence of different land uses on soil
479 organic matter quality and quantity (Cross et al. 2011). We believe that understanding the
480 impact of tillage on priming effect induced by PyOM is specially important in the context of
481 biochar application. However this aspect was never investigated in the field, although its
482 impact was simulated in incubation experiments by mixing of soil (Kuzyakov et al., 2009).

483 We believe that these two aspects are crucial to understand the impact of PyOM induced
484 priming effect on soil C budget.

Conclusions

We found that on average PyOM induces a priming effect of similar magnitude but opposite direction on native soil organic matter (positive priming effect) and fresh organic matter (negative priming effect). The priming effect on native soil organic matter was found to be related with time and PyOM-C content, with the positive priming effect occurring mostly on the short term and induced by PyOM characterized by a low C content, and negative PE appearing at a second stage.

We discussed the different mechanisms that can be involved in the PyOM induced priming effect on the native soil organic matter. We believe that the presence of a labile fraction in PyOM may induce a positive priming effect on the short term by triggering the activity of soil microorganisms. Simultaneously PyOM may promote the physical protection of organic matter by sorption on PyOM surfaces or into microaggregates, however the effect of this mechanism appears only in a second stage when positive priming effect has ceased.

We conclude that, although many uncertainties still exist, particularly on the parameters driving the amplitude and the direction of priming effect, adding PyOM to the soil induces a cumulative positive priming effect on a yearly time scale, on the native soil organic matter, which may be counterbalanced by the negative priming effect observed on fresh organic matter. However, further investigations on the factors influencing the priming effect induced on fresh organic matter are required; particularly studies employing double isotopic labelling would allow the determination of the priming effect both on the fresh and on the native soil organic matter.

Acknowledgments

508 We are grateful to three anonymous reviewers who carefully read the manuscript and
509 provided insightful comments.

510

511 **Tables**

512 Table 1: Observations of priming on the native soil organic matter, for the study without isotopes the PE column reflect whether PyOM addition
513 induced changes in soil respiration rather than in native soil organic matter mineralization. Only studies employing isotopes were then included in
514 the meta-analysis.

Author	**Type of experiment**	**Soil type**	**Biochar type**	**Climate**	**PE direction**	**Mechanism proposed**
(Bell & Worrall, 2011)	Lysimeter in the field without isotope	1)arable soil (Sandy clay loam), vegetated and unvegetated 2)Black humified peat/organic	Lump wood (C content approx. 80%)	Temperate	Mostly **Neutral** in the unvegetated treatment **Positive** in 2 out of 9 treatments.	Short-term increase of microbial biomass due to increased habitability (foci hypotheses).

(Cheng *et al.*, 2008)	Incubation of soil sampled next to a furnace with an adjacent, no isotopes (unknown quantity of PyOM in the soil)	Broad variety of Canadian soils	Charcoal was produced in furnaces	Gradient of soils	**Negative**	
(Cross & Sohi, 2011)	Incubation, with isotopes	Silty-clay loam 1)bare (C content 1%) 2)Arable C content(1.9%) 3)grassland (C content 3.6 %)	Range of sugar bagasse with different charring temperature (350-550 °C) and time (20-80)	Temperate	**Neutral** or **negative** when calculated on a C basis	Sorption, particularly of readily available substrates

(Jones *et al.*, 2011)	Incubation with isotopes	Cambisol	Mix of wood	Temperate	**Negative**	Several hypotheses, among them: 1)Sorption, 2)Enzymes sorption 3) liming effect
(Keith *et al.*, 2011)	Incubation with isotopes	Clay, rich in smectite, Vertisol	Eucalyptus Salinga	Queensland Australia	**Positive**, particularly in the first 18 days for SOM	Labile content of PyOM promote mineralization of native soil organic matter,ko
(Kolb *et al.*, 2009)	Incubation no isotopes	Several types	Mix of manure and pine wood	4 different soils series	**Positive**	Increase in microbial biomass responsible for the PE

						(foci). The strongest PE comes from the more fertile soils
(Kuzyakov *et al.*, 2009)	Incubation using stable isotopes	Loess soil and Loess	Grass L. perenne (400°C)	Temperate	**Neutral** (in loess derived soil) or **negative** (in Loess)	In Loess sorption of nutrients and organic C
(Luo *et al.*, 2011)	Incubation with isotopes	Aquic Paleudalf (Silty loam)	Miscantus giganteus	Temperate	**Positive** PE, relatively higher in the low pH soil, higher from PyOM of	Co-metabolism, due to the high concentration of available (i.e. dissolved) C

					350 °C	present in biochar. Moreover the 350 induced more priming than the 700. Relative higher PE in low pH than in high pH (liming induced PE).
(Maestrini *et al.*, 2014a)	Incubation with isotopes	Cambisol	Rye grass	Temperate	**Positive** in the first 18 days **negative** from day 18 to day 158	Co-metabolism and sorption of organic matter.

(Major *et al.*, 2010)	Field experiment with plants, with isotopes	Savanna Oxisol	Mangifera Indica (500 °C)	Tropical	**Positive**	PyOM induced more plant growth resulting in higher autotrphic respiration.
(Novak *et al.*, 2010)	Incubation without isotopes	Loamy sandy	Shell pecan	Subtropical	**Neutral**	
(Santos *et al.*, 2012)	Incubation with isotopes	Sandy loam and salty silt loam	Pinus ponderosa	Mediterranean	**Neutral**	
(Zimmerman *et al.*, 2011)	Incubation with isotopes	Several soil types	Grass and wood	Subtropical	**Positive** in the first 90 days and **negative** afterward	Co-metabolism in the beginning and encapsulation

						and/or organic matter sorption ob biochar surface.
(Singh *et al.*, 2012b)	Incubation with isotopes	Clay	Several (wood/leaf/manure, 400-550°C, under N_2, activated or not activated).	Queensland (Australia)	**Positive** in the first two years, and **negative** afterward.	
(Farrell *et al.*, 2013)	Incubation with isotopes	Coarse texture	Wheat and Eucalipt shoots	Temperate Mediterranean	**Positive**	Co-metabolism
(Kimetu *et al.*, 2009)	Incubation without isotopes	Clay	Wood (450°C) eucalyptus salinga	Tropical	**Negative** in the low C soil and no difference in soil	PyOM sorbs dissolved organic C or PyOM promotes

					respiration in the high C soil.	aggregation

515

516

517 Table 2: Studies investigating the PE on the fresh organic matter. The column entitled “Formula for PE” indicates whether equation 4 or equation 3
518 was used to measure PE. Equation 4 was used when it was not possible to discriminate between the CO_2 derived from PyOM and the CO_2 derived
519 from fresh organic matter.

Author	**Type of experiment**	**Soil type**	**PyOM characteristics type**	**Co-substrate**	**Climate**	**Formula for PE**	**Mechanism proposed**	**PE direction**
(Abiven & Andreoli, 2010)	Incubation without isotopes	Cambisol with 30% clay, 4% organic C and pH 6.1	Picea abies, 450 °C 5 h anoxic	Four different co-substrates with a range of material	Temperate	Equation 4		**Neutral**
(Hamer *et al.*, 2004)	Incubation with isotopes	Quartz sand	Maize, Rice, Straw	Glucose	Temperate	Equation 3	Co-metabolism	**Positive**
(Zavalloni *et al.*, 2011)	Incubation without isotopes	Silt-loam Cambisol	Wood	Wheat-straw	Temperate	Equation 4		**Negative**
(Jones *et*	Incubation with	Sandy clay	Wood	Rye grass and	Temperate	Equation 3		**Negative** for

al., 2012)	isotopes	loam		glucose, aminoacids cocktail				rye grass, **neutral** for other substrate
(Keith *et al.*, 2011)	Incubation with isotopes	Clay, Vertisol	Eucalyptus Salinga	Sugar Cane residue	Subtropical	Equation 3	PyOM promotes incorporation of fresh organic matter in aggregates	**Negative**
(Liang *et al.*, 2010)	Incubation with isotopes. Terra preta (soils containing old PyOM) contrasted to adjacent soil (poor in PyOM)	Anthrosol	Already present in the soil		Tropical	Equation 3	Fresh organic matter incorporation in the Anthrosol rich in PyOM was higher than in the adjacent soil (poor in PyOM), but this did not result in a decrease of mineralization.	**Neutral**.
(Wardle *et al.*, 2008)	Litter bag, without isotopes		Empetrum hermaphroditum	Forest humus	Boreal	Not used	The “foci hypotheses”	**Positive**

520

521

522

523

524 ## References

525 Abiven S, Andreoli R (2010) Charcoal does not change the decomposition rate of mixed
526 litters in a mineral cambisol: a controlled conditions study. *Biology and Fertility of Soils*,
527 **47**, 111–114.
528 Abiven S, Hengartner P, Schneider M, Singh N, Schmidt MWI (2011) Pyrogenic carbon
529 soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. *Soil*
530 *Biology and Biochemistry*, **43**, 1615–1617.
531 Bailey VL, Fansler SJ, Smith JL, Bolton H (2011) Reconciling apparent variability in effects
532 of biochar amendment on soil enzyme activities by assay optimization. *Soil Biology and*
533 *Biochemistry*, **43**, 296–301.
534 Bell MJ, Worrall F (2011) Charcoal addition to soils in NE England: a carbon sink with
535 environmental co-benefits? *The Science of the Total Environment*, **409**, 1704–14.
536 Bingeman CW, Varner JE, Martin WP (1953) The effect of the Addition of Organic Materials
537 on the Decomposition of an Organic Soil. *Soil Science Society Proceedings*, **17**, 34–38.
538 Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and
539 their dependence on soil microbial biomass and community structure: critical review.
540 *Biology and Fertility of Soils*, **45**, 115–131.
541 Blagodatskaya EV, Blagodatsky S a., Anderson T-H, Kuzyakov Y (2007) Priming effects in
542 Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied*
543 *Soil Ecology*, **37**, 95–105.
544 Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y (2011) Three-source-partitioning
545 of microbial biomass and of CO2 efflux from soil to evaluate mechanisms of priming
546 effects. *Soil Biology and Biochemistry*, **43**, 778–786.
547 Blagodatsky S, Blagodatskaya E, Yuyukina T, Kuzyakov Y (2010) Model of apparent and real
548 priming effects: Linking microbial activity with soil organic matter decomposition. *Soil*
549 *Biology and Biochemistry*, **42**, 1275–1283.
550 Bornemann LC, Kookana RS, Welp G (2007) Differential sorption behaviour of aromatic
551 hydrocarbons on charcoals prepared at different temperatures from grass and wood.
552 *Chemosphere*, **67**, 1033–42.
553 Brodowski S, John B, Flessa H, Amelung W (2006) Aggregate-occluded black carbon in soil.
554 *European Journal of Soil Science*, **57**, 539–546.
555 Bruun S, Jensen ES, Jensen LS (2008) Microbial mineralization and assimilation of black
556 carbon: Dependency on degree of thermal alteration. *Organic Geochemistry*, **39**, 839–
557 845.
558 Chen B, Zhou D, Zhu L (2008) Transitional Adsorption and Partition of Nonpolar and Polar
559 Aromatic Contaminants by Biochars of Pine Needles with Different Pyrolytic
560 Temperatures. *Environmental science & technology*, **42**, 5137–5143.
561 Cheng C-H, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black
562 carbon by biotic and abiotic processes. *Organic Geochemistry*, **37**, 1477–1488.
563 Cheng C-H, Lehmann J, Thies JE, Burton SD (2008) Stability of black carbon in soils across
564 a climatic gradient. *Journal of Geophysical Research*, **113**, 1–10.
565 Chun Y, Sheng G, Chiou CT, Xing B (2004) Compositions and Sorptive Properties of Crop
566 Residue-Derived Chars. *Environmental science & technology*, **38**, 4649–4655.

567 Conde E, Cardenas M, Poncemendoza A, Lunaguido M, Cruzmondragon C, Dendooven L
568 (2005) The impacts of inorganic nitrogen application on mineralization of C-labelled
569 maize and glucose, and on priming effect in saline alkaline soil. *Soil Biology and*
570 *Biochemistry*, **37**, 681–691.
571 Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Koelmans AA, Van Noort PCM
572 (2005a) Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen
573 in Sediments and Soils: Mechanisms and Consequences for Distribution,
574 Bioaccumulation, and Biodegradation. *Environmental Science & Technology*, **39**, 6881–
575 6895.
576 Cornelissen G, Haftka J, Parsons J, Gustafsson O (2005b) Sorption to black carbon of organic
577 compounds with varying polarity and planarity. *Environmental Science & Technology*,
578 **39**, 3688–94.
579 Crawley MJ (2007) *The R book*. Wiley, Chichester.
580 Cross A, Sohi SP (2011) The priming potential of biochar products in relation to labile carbon
581 contents and soil organic matter status. *Soil Biology and Biochemistry*, **43**, 2127–2134.
582 Crow SE, Lajtha K, Bowden RD, Yano Y, Brant JB, Caldwell BA, Sulzman EW (2009)
583 Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-
584 growth forest. *Forest Ecology and Management*, **258**, 2224–2232.
585 Crutzen PJ, Andreae MO (1990) Biomass burning in the tropics: impact on atmospheric
586 chemistry and biogeochemical cycles. *Science*, **250**, 1669–78.
587 Fabbri D, Torri C, Spokas K a. (2012) Analytical pyrolysis of synthetic chars derived from
588 biomass with potential agronomic application (biochar). Relationships with impacts on
589 microbial carbon dioxide production. *Journal of Analytical and Applied Pyrolysis*, **93**,
590 77–84.
591 Farrell M, Kuhn TK, Macdonald LM *et al.* (2013) Microbial utilisation of biochar-derived
592 carbon. *The Science of the Total Environment*, **465**, 288–297.
593 Fellet G, Marchiol L, Delle Vedove G, Peressotti a (2011) Application of biochar on mine
594 tailings: effects and perspectives for land reclamation. *Chemosphere*, **83**, 1262–7.
595 Flannigan M, Cantin AS, De Groot WJ, Wotton M, Newbery A, Gowman LM (2013) Global
596 wildland fire season severity in the 21st century. *Forest Ecology and Management*, **294**,
597 54–61.
598 Fontaine S, Barot S (2005) Size and functional diversity of microbe populations control plant
599 persistence and long-term soil carbon accumulation. *Ecology Letters*, **8**, 1075–1087.
600 Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of
601 microbial competition? *Soil Biology and Biochemistry*, **35**, 837–843.
602 Gianfreda L, Rao MA (2011) Stabilizing Enzymes as Synthetic Complexes. In: *Methods of*
603 *soil enzymology* (ed Dick R), pp. 319–369. Soil Science Society of America, Madison,
604 WI.
605 Hamer U, Marschner B (2005) Priming effects in soils after combined and repeated substrate
606 additions. *Geoderma*, **128**, 38–51.
607 Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon
608 and glucose mineralisation. *Organic Geochemistry*, **35**, 823–830.
609 Hammes K, Smernik RJ, Skjemstad JO, Herzog A, Vogt UF, Schmidt MWI (2006) Synthesis
610 and characterisation of laboratory-charred grass straw (Oryza sativa) and chestnut wood
611 (Castanea sativa) as reference materials for black carbon quantification. *Organic*
612 *Geochemistry*, **37**, 1629–1633.
613 Harvey OR, Herbert BE, Rhue RD, Kuo L-J (2011) Metal interactions at the biochar-water
614 interface: energetics and structure-sorption relationships elucidated by flow adsorption
615 microcalorimetry. *Environmental Science & Technology*, **45**, 5550–6.

616 Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural changes
617 during the initial phase of microbial degradation of pyrogenic plant residues in soil.
618 *Organic Geochemistry*, **40**, 332–342.
619 Ingham RE, Trofymov JA, Ingham ER, Coleman DC (1985) Interactions of Bacteria, Fungi,
620 and their Nematode Grazers: Effects on Nutrient Cycling and Plant Growth. *Ecological*
621 *Monographs*, **55**, 119–140.
622 James G, Sabatini D a, Chiou CT, Rutherford D, Scott AC, Karapanagioti HK (2005)
623 Evaluating phenanthrene sorption on various wood chars. *Water research*, **39**, 549–58.
624 Jin H (2010) *Characterization of microbial life colonizing biochar and biocharamended soils.*
625 Cornell University, Ithaka, NY.
626 Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, DeLuca TH (2011) Short-
627 term biochar-induced increase in soil CO2 release is both biotically and abiotically
628 mediated. *Soil Biology and Biochemistry*, **43**, 1723–1731.
629 Jones DL, Rousk J, Edwards-Jones G, DeLuca TH, Murphy DV (2012) Biochar-mediated
630 changes in soil quality and plant growth in a three year field trial. *Soil Biology and*
631 *Biochemistry*, **45**, 113–124.
632 Joseph SD, Camps-Arbestain M, Lin Y *et al.* (2010) An investigation into the reactions of
633 biochar in soil. *Australian Journal of Soil Research*, **48**, 501.
634 Kaiser K, Guggenberger G (2000) The role of DOM sorption to mineral surfaces in the
635 preservation of organic matter in soils. *Organic Geochemistry*, **31**, 711–725.
636 Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on dynamics of
637 dissolved organic matter in soil: a review. *Soil Science*, **165**, 277–304.
638 Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic molecular structure of plant
639 biomass-derived black carbon (biochar). *Environmental Science & Technology*, **44**,
640 1247–53.
641 Keith A, Singh B, Singh BP (2011) Interactive priming of biochar and labile organic matter
642 mineralization in a smectite-rich soil. *Environmental Science & Technology*, **45**, 9611–8.
643 Kimetu JM, Lehmann J (2010) Stability and stabilisation of biochar and green manure in soil
644 with different organic carbon contents. *Australian Journal of Soil Research*, **48**, 577.
645 Kimetu JM, Lehmann J, Kinyangi JM, Cheng CH, Thies J, Mugendi DN, Pell a. (2009) Soil
646 organic C stabilization and thresholds in C saturation. *Soil Biology and Biochemistry*, **41**,
647 2100–2104.
648 Knicker H (2010) "Black nitrogen" – an important fraction in determining the recalcitrance of
649 charcoal. *Organic Geochemistry*, **41**, 947–950.
650 Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of Charcoal Quantity on Microbial
651 Biomass and Activity in Temperate Soils. *Soil Science Society of America Journal*, **73**,
652 1173.
653 Kuhlbusch TAJ, Crutzen PJ (1995) Toward a global estimate of black carbon in residues of
654 vegetation fires representing a sink of atmospheric CO2 and a source of 02. *Global*
655 *Biogeochemical Cycles*, **9**, 491–501.
656 Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. *Soil*
657 *Biology and Biochemistry*, **42**, 1363–1371.
658 Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X (2009) Black carbon decomposition
659 and incorporation into soil microbial biomass estimated by 14C labeling. *Soil Biology*
660 *and Biochemistry*, **41**, 210–219.
661 De Lafontaine G, Asselin H (2011) Soil charcoal stability over the Holocene across boreal
662 northeastern North America. *Quaternary Research*, **76**, 196–200.
663 Lehmann J, Joseph S (eds.) (2009) *Biochar for environmental managers*. Earthscan, London.

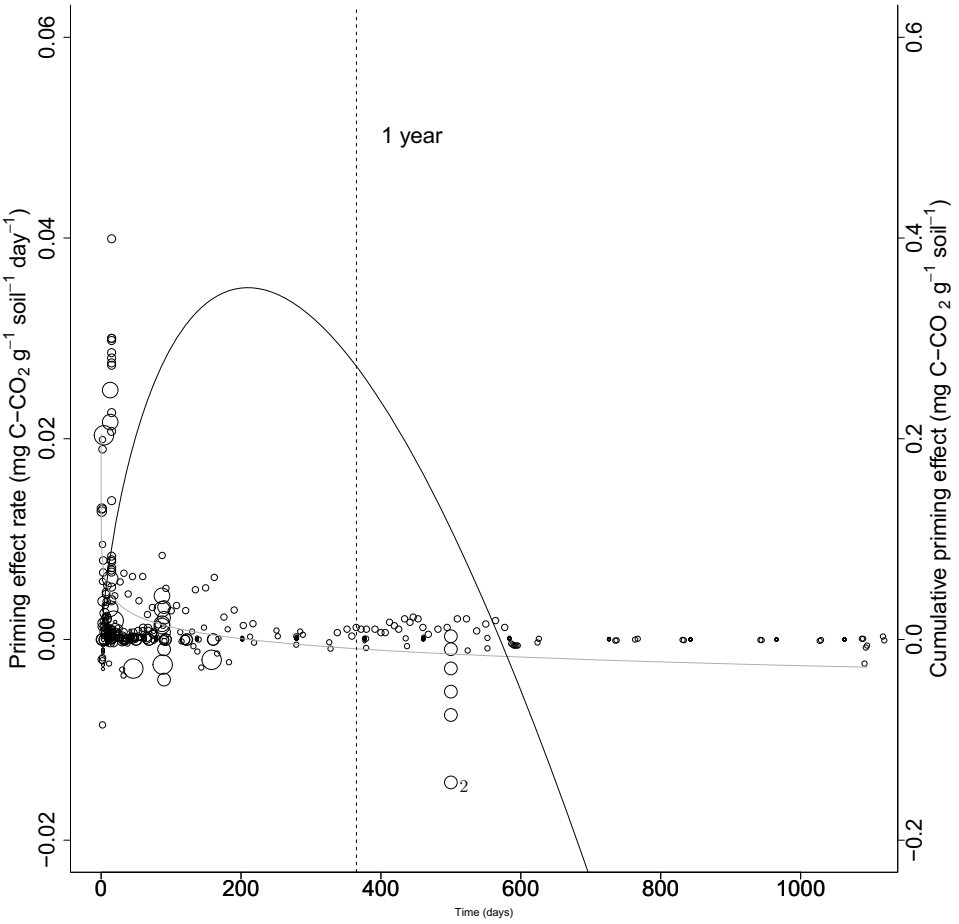
664 Lehmann J (2007) Bio-energy in the black. *Frontiers in Ecology and the Environment*, **5**,
665 381–387.
666 Lehmann J, Liang B, Solomon D *et al.* (2005) Near-edge X-ray absorption fine structure
667 (NEXAFS) spectroscopy for mapping nano-scale distribution of organic carbon forms in
668 soil: Application to black carbon particles. *Global Biogeochemical Cycles*, **19**.
669 Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar
670 effects on soil biota – A review. *Soil Biology and Biochemistry*, **43**, 1812–1836.
671 Liang B, Lehmann J, Sohi SP *et al.* (2010) Black carbon affects the cycling of non-black
672 carbon in soil. *Organic Geochemistry*, **41**, 206–213.
673 Liu XH, Zhang XC (2012) Effect of Biochar on pH of Alkaline Soils in the Loess Plateau:
674 Results from Incubation Experiments. *International Journal of Agriculture and Biology*,
675 **14**, 745–750.
676 Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes PC (2011) Short term soil priming
677 effects and the mineralisation of biochar following its incorporation to soils of different
678 pH. *Soil Biology and Biochemistry*, **43**, 2304–2314.
679 Lynch JA, Clark JS, Stocks BJ (2004) Charcoal production, dispersal, and deposition from the
680 Fort Providence experimental fire: interpreting fire regimes from charcoal records in
681 boreal forests. *Canadian Journal of Forest Research*, **34**, 1642.
682 Maestrini B, Herrmann AM, Nannipieri P, Schmidt MWI, Abiven S (2014a) Ryegrass-derived
683 pyrogenic organic matter changes organic carbon and nitrogen mineralization in a
684 temperate forest soil. *Soil Biology and Biochemistry*, **69**, 291–301.
685 Maestrini B, Abiven S, Singh N, Bird J, Torn MS, Schmidt MWI (2014b) Carbon losses from
686 pyrolysed and original wood in a forest soil under natural and increased N deposition.
687 *Biogeosciences Discussions*, **11**, 1–31.
688 Major J, Lehmann J, Rondon M, Goodale C (2010) Fate of soil-applied black carbon:
689 downward migration, leaching and soil respiration. *Global Change Biology*, **16**, 1366–
690 1379.
691 Moritz M, Parisien M, Batllori E, Krawchuk MA, Van Dorn J, Ganz DJ, Hayhoe K (2012)
692 Climate change and disruptions to global fire activity. *Ecosphere*, **3**, 1–22.
693 Nannipieri P, Gianfreda L (1998) Kinetics of enzyme reactions in soil environments. In:
694 *Structures and reactions of soil particles* (eds Huang PM, Senesi N, Buffle J). Wiley,
695 New York.
696 Nannipieri P, Giagnoni L, Renella G *et al.* (2012) Soil enzymology: classical and molecular
697 approaches. *Biology and Fertility of Soils*, **48**, 743–762.
698 Novak JM, Busscher WJ, Watts DW, Laird D a., Ahmedna M a., Niandou M a. S (2010)
699 Short-term CO2 mineralization after additions of biochar and switchgrass to a Typic
700 Kandiudult. *Geoderma*, **154**, 281–288.
701 Piccolo a., Pietramellara G, Mbagwu JSC (1997) Use of humic substances as soil conditioners
702 to increase aggregate stability. *Geoderma*, **75**, 267–277.
703 Pietikäinen J, Kiikkilä O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on
704 the microbial community of the underlying humus. *Oikos*, **89**, 231–242.
705 Preston CM, Schmidt MWI (2006) Black (pyrogenic) carbon: a synthesis of current
706 knowledge and uncertainties with special consideration of boreal regions.
707 *Biogeosciences*, **3**, 397–420.
708 Redfield RJ (2002) Is quorum sensing a side effect of diffusion sensing? *Trends in*
709 *Microbiology*, **10**, 365–70.
710 Rumpel C, Chaplot V, Planchon O, Bernadou J, Valentin C, Mariotti A (2006) Preferential
711 erosion of black carbon on steep slopes with slash and burn agriculture. *Catena*, **65**, 30–
712 40.

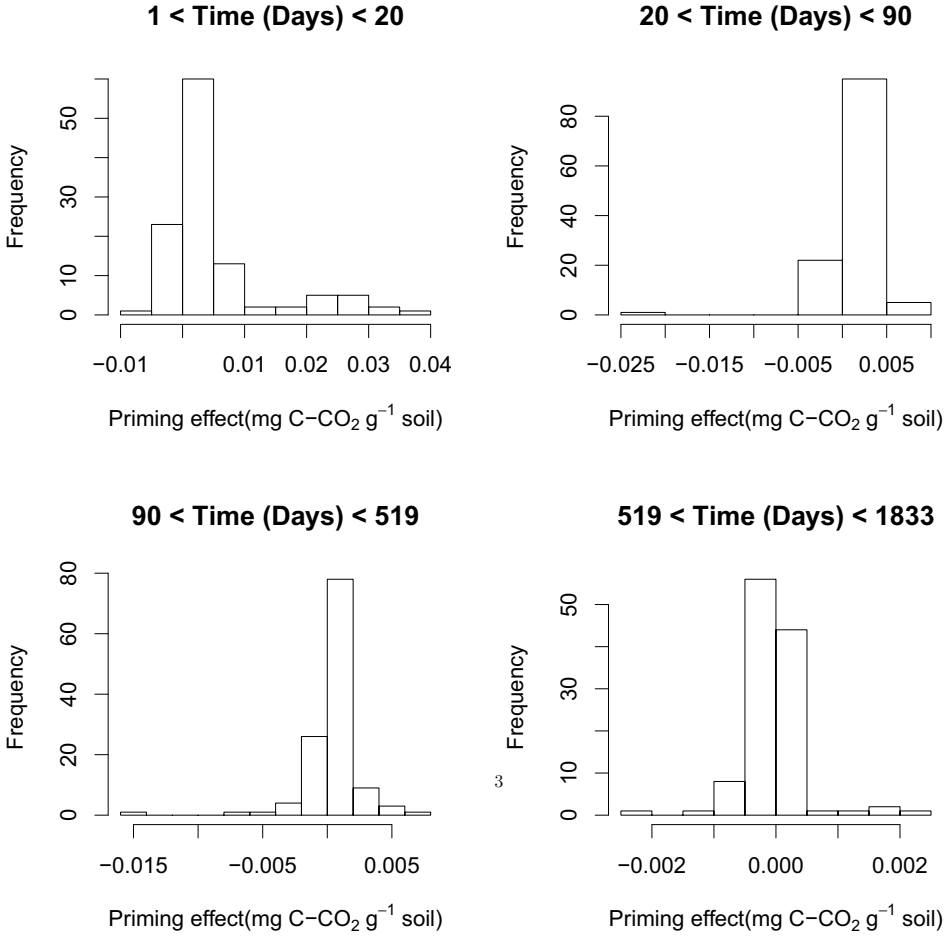
713 Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in
714 temperate forest soils. *Soil Biology and Biochemistry*, **51**, 115–124.
715 Sayer EJ, Heard MS, Grant HK, Marthews TR, Tanner EVJ (2011) Soil carbon release
716 enhanced by increased tropical forest litterfall. *Nature Climate Change*, **1**, 304–307.
717 Schmidt MWI, Noack A. (2000) Black carbon in soils and sediments: Analysis, distribution,
718 implications, and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.
719 Schneider MPW, Lehmann J, Schmidt MWI (2011) Charcoal quality does not change over a
720 century in a tropical agro-ecosystem. *Soil Biology and Biochemistry*, **43**, 1992–1994.
721 Singh N, Abiven S, Torn MS, Schmidt MW. (2012a) Fire-derived organic carbon turnover in
722 soils on a centennial scale. *Biogeoscience*, **9**, 1–11.
723 Singh BP, Cowie AL, Smernik RJ (2012b) Biochar carbon stability in a clayey soil as a
724 function of feedstock and pyrolysis temperature. *Environmental Science & Technology*,
725 **46**, 11770–8.
726 Singh N, Abiven S, Maestrini B, Bird J a., Torn MS, Schmidt MWI (2013) Transformation
727 and stabilization of pyrogenic organic matter in a temperate forest field experiment.
728 *Global Change Biology*, doi:10.1111/gcb.12459.
729 Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and Fungal Contributions to Carbon
730 Sequestration in Agroecosystems. *Soil Science Society of America Journal*, **70**, 555.
731 Skjemstad JO, Janik LJ, Head MJ, McClure SG (1993) High energy ultraviolet photo-
732 oxidation: a novel technique for studying physically protected organic matter in clay-
733 and silt-sized aggregates. *Journal of Soil Science*, **44**, 485–499.
734 Sollins P, Homann P, Caldwell B a. (1996) Stabilization and destabilization of soil organic
735 matter: mechanisms and controls. *Geoderma*, **74**, 65–105.
736 Spokas KA (2010) Review of the stability of biochar in soils: predictability of O:C molar
737 ratios. *Carbon Management*, **1**, 289–303.
738 Steiner C, Das KC, Garcia M, Förster B, Zech W (2008a) Charcoal and smoke extract
739 stimulate the soil microbial community in a highly weathered xanthic Ferralsol.
740 *Pedobiologia*, **51**, 359–366.
741 Steiner C, Glaser B, Geraldes Teixeira W, Lehmann J, Blum WEH, Zech W (2008b) Nitrogen
742 retention and plant uptake on a highly weathered central Amazonian Ferralsol amended
743 with compost and charcoal. *Journal of Plant Nutrition and Soil Science*, **171**, 893–899.
744 Stewart CE, Zheng J, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar
745 mitigates green-house gas emissions and increases carbon sequestration in temperate
746 soils. *GCB Bioenergy*, **5**, 153–164.
747 Théry-Parisot I, Chabal L, Chrzavzez J (2010) Anthracology and taphonomy, from wood
748 gathering to charcoal analysis. A review of the taphonomic processes modifying charcoal
749 assemblages, in archaeological contexts. *Palaeogeography, Palaeoclimatology,*
750 *Palaeoecology*, **291**, 142–153.
751 Vasilyeva N a., Abiven S, Milanovskiy EY, Hilf M, Rizhkov O V., Schmidt MWI (2011)
752 Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter
753 depletion in a Chernozem. *Soil Biology and Biochemistry*, **43**, 1985–1988.
754 Verheijen F, Jeffery CSA, Van der Velde M, Diafas I (2010) *Biochar Application to Soils: A*
755 *Critical Scientific Review of Effects on Soil Properties Processes and Functions*.
756 Technical report Joint Research Commission of the European Commission,
757 Luxembourg.
758 Wallenstein MD, Burns RG (2011) Ecology of extracellular enzymes activities and organic
759 matter degradation in soil. In: *Methods of soil enzymology2* (ed Dick RP), pp. 35–55.
760 Soil Science Society of America.

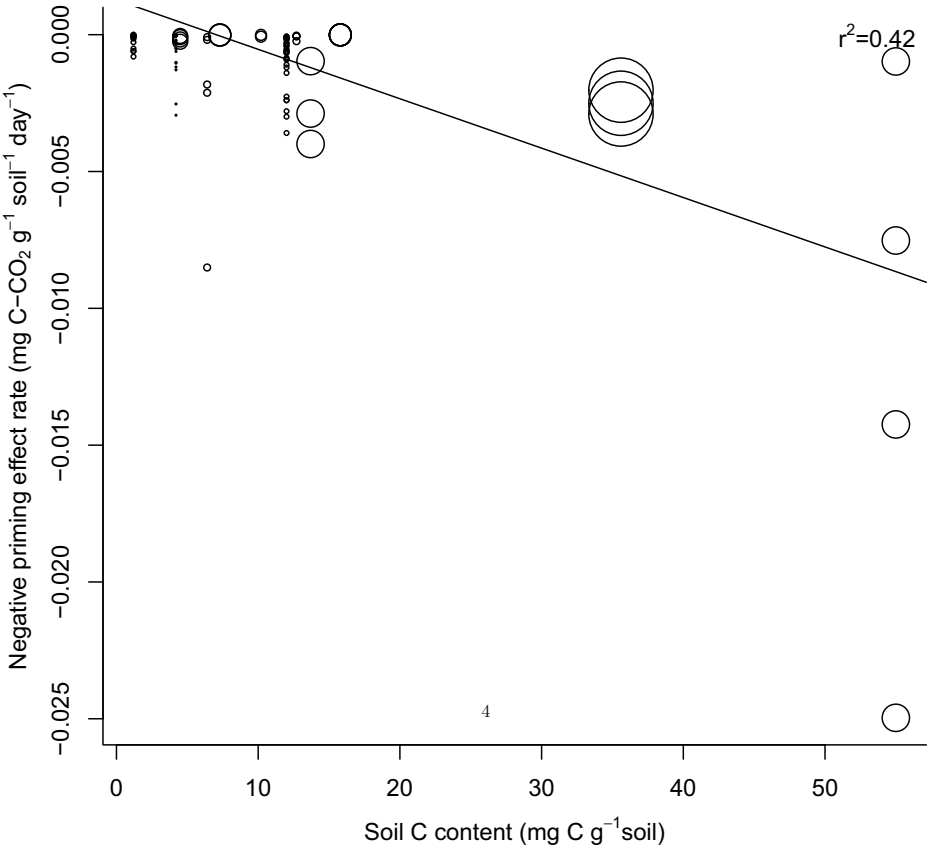
761 Wardle DA, Nilsson M-C, Zackrisson O (2008) Fire-derived charcoal causes loss of forest
762 humus. *Science*, **320**, 629.
763 Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in
764 soil – concepts and mechanisms. *Plant and Soil*, **300**, 9–20.
765 Woolf D, Lehmann J (2012) Modelling the long-term response to positive and negative
766 priming of soil organic carbon by black carbon. *Biogeochemistry*, **111**, 83–95.
767 Wu J, Brookes PC, Jenkinson DS (1993) Formation and destruction of microbial biomass
768 during the decomposition of glucose and ryegrass in soil. *Soil Biology and Biochemistry*,
769 **25**, 1435–1441.
770 Zackrisson O, Nilsson M, Wardle DA (1996) Key Ecological Function of Charcoal from
771 Wildfire in the Boreal Forest Key ecological function of charcoal from wildfire in the
772 Boreal forest. *Oikos*, **77**, 10–19.
773 Zavalloni C, Alberti G, Biasiol S, Vedove GD, Fornasier F, Liu J, Peressotti A (2011)
774 Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study.
775 *Applied Soil Ecology*, **50**, 45–51.
776 Zimmerman AR, Gao B (2013) The stability of biochar in the environment. In: *Biochar and*
777 *soil biota* (eds Ladygina N, Rineau F), pp. 1–40. CRC press, Boca Raton.
778 Zimmerman AR, Gao B, Ahn M-Y (2011) Positive and negative carbon mineralization
779 priming effects among a variety of biochar-amended soils. *Soil Biology and*
780 *Biochemistry*, **43**, 1169–1179.
781 Van Zwieten L, Kimber S, Morris S *et al.* (2009) Effects of biochar from slow pyrolysis of
782 papermill waste on agronomic performance and soil fertility. *Plant and Soil*, **327**, 235–
783 246.
784
785

Figure captions

Figure 1: Priming effect as a function of time. The grey line represent the modelled rate of priming effect, while the black line represent the cumulative modelled priming effect, and is referred to the right y-axis. The size of the points is proportional to the weight of each case calculated using equation 1.
Figure 2: Frequency distribution of priming effect in the time quartiles of the database.
Figure 3: Negative priming effect as a function of soil C content.The size of the points is proportional to the weight of each case calculated using equation 1.


1 year
Priming effect rate (mg C−CO2 g−1 soil−1 day−1)
Cumulative priming effect (mg C−CO 2 g−1 soil−1)
Time (days)
0.06
0.04
0.02
0.00
−0.02
0.6
0.4
0.2
0.0
−0.2
0
200
400
600
800
1000
2


1 < Time (Days) < 20
Frequency
0
10
30
50
−0.01
0.01
0.02
0.03
0.04
Priming effect(mg C−CO2 g−1 soil)
20 < Time (Days) < 90
Frequency
0
20
40
60
80
−0.025
−0.015
−0.005
0.005
Priming effect(mg C−CO2 g−1 soil)
90 < Time (Days) < 519
Frequency
0
20
40
60
80
−0.015
−0.005
0.005
Priming effect(mg C−CO2 g−1 soil)
519 < Time (Days) < 1833
Frequency
0
10
30
50
−0.002
0.000
0.002
Priming effect(mg C−CO2 g−1 soil)


r2=0.42
Negative priming effect rate (mg C−CO2 g−1 soil−1 day−1)
0.000
−0.005
−0.010
−0.015
−0.020
−0.025
0
10
20
30
40
50
Soil C content (mg C g−1soil)

4 Manuscript IV

Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment.

Nimisha Singh, Samuel Abiven, Bernardo Maestrini, Jeffrey A. Bird, Margaret S. Torn and Michael W. I. Schmidt

The manuscript was published online in Global Change Biology (doi: 10.1111/gcb.12459)

Candidate contribution: The candidate maintained the field experiment, contributed to manuscript, and data interpretation.

Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment

Nimisha Singh[1], Samuel Abiven[1], Bernardo Maestrini[1], Jeffrey A. Bird[2], Margaret S. Torn[3] and Michael W. I. Schmidt[1]*

[1] Department of Geography, University of Zurich, Winterthurerstrasse 190, Zürich 8057, Switzerland

[2] School of Earth and Environmental Sciences, Queens College, City University of New York, Flushing, New York 11367

[3]Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

*Corresponding author:

samuel.abiven@geo.uzh.ch

Phone +41 44 6355183; Fax +41 44 6356841

nimisha.nimisha@geo.uzh.ch

bernardo.maestrini@geo.uzh.ch

jbird@qc.cuny.edu

mstorn@lbl.gov

michael.schmidt@geo.uzh.ch

Paper type: Primary Research Article

Abstract

Pyrogenic organic matter (PyOM) decomposes on centennial timescale in soils, but the processes regulating its decay are poorly understood. We conducted one of the first studies of PyOM and wood decomposition in a temperate forest using isotopically labeled organic substrate, and quantified microbial incorporation and physico-chemical transformations of PyOM in situ. Stable-isotope (^{13}C and ^{15}N) enriched PyOM and its precursor wood were added to the soil at 2 cm depth at ambient (N0) and increased (N+) levels of nitrogen fertilization. The carbon (C) and nitrogen (N) of added PyOM or wood were tracked through soil to 15 cm depth, in physically separated soil density fractions and in benzene polycarboxylic acids (BPCA) molecular markers. After 10 months in situ, more PyOM-derived C (>99% of initial ^{13}C-PyOM) and N (90% of initial ^{15}N-PyOM) was recovered than wood derived C (48% of ^{13}C-wood) and N (89% under N0 and 48% under N+). PyOM-C and wood-C migrated at the rate of 126 mm yr^{-1} with 3-4% of PyOM-C and 4-8% of wood-C recovered below the application depth. Most PyOM C was recovered in the free light fraction (fLF) (74%), with 20% in aggregate-occluded and 6% in mineral associated fractions —fractions that typically have much slower turnover times. In contrast, wood C was recovered mainly in occluded (33%) or dense fraction (27%). PyOM addition induced loss of native C from soil (priming effect), particularly in fLF (13%). The total BPCA-C content did not change but after ten months the degree of aromatic condensation of PyOM decreased, as determined by relative contribution of benzene hexa-carboxylic acid (B6CA) to the total BPCA C. Soil microbial biomass (SMB) assimilated 6-10% of C from the wood, while PyOM contributions was negligible

(0.14–0.18%). The addition of N had no effect on the dynamics of PyOM while limited effect on wood.

Keywords: pyrogenic organic matter, benzene polycarboxylic acids, density fractionation, priming effect, soil organic matter, nitrogen deposition, ^{13}C, ^{15}N, microbial biomass

Abbreviations: PyOM = Pyrogenic organic matter; BPCA= Benzene polycarboxylic acids; fLF = Free light fraction; oLF = Occluded light fraction; DF= Dense fraction; SOM = Soil organic matter; PE = Priming effect; SMB = Soil microbial biomass.

69 **1. Introduction**

70

71 Pyrogenic organic matter (PyOM), a product of incomplete combustion of biomass
72 (Goldberg, 1985), is ubiquitous in soils (Schmidt & Noack, 2000) and can account for up
73 to 45% of total soil organic carbon (SOC) (Bird *et al.*, 1999, Lehmann *et al.*, 2008,
74 Rovira *et al.*, 2009, Schmidt *et al.*, 1999b, Skjemstad *et al.*, 2002). In recent years, PyOM
75 has received considerable interest by researchers, in part, because of its potential
76 relevance to the C cycle of terrestrial ecosystem (Schmidt *et al.*, 2011). Climate change
77 projections predict an increase in the wildfire frequency and intensity in temperate and
78 boreal regions (Moritz *et al.*, 2012, Westerling *et al.*, 2006), which would increase the
79 inputs of PyOM to soils. The addition of PyOM to soils could also constitute a method
80 for sequestering C (DeLuca & Aplet, 2008, Lehmann *et al.*, 2006), if its residence time
81 in soil were sufficiently longer than its precursor biomass. However, many uncertainties
82 remain about the dynamics of PyOM C and N in soils including *in situ* PyOM turnover
83 rates, degradation pathways and stabilization mechanisms (Knicker, 2011, Singh *et al.*,
84 2012).

85

86 Most wood decomposition studies estimate decay rates as either mass loss or density
87 change per unit time (Chambers *et al.*, 2001). These studies have estimated yearly to
88 decadal turnover time for fine woody debris and litter (Abbott & Crossley, 1982, Busse,
89 1994, Clark *et al.*, 2002, Fasth *et al.*, 2011, Guo *et al.*, 2006, Zell *et al.*, 2009).
90 Degradation rates of pyrolysed wood is slower than its initial precursor biomass and
91 becomes even slower with increasing pyrolysis temperatures (Baldock & Smernik,

2002). The slow decomposition of pyrolysed wood, and pyrogenic matter in general, has been attributed to changes in physical and chemical structure, including an increase in the degree of aromatic condensation as the pyrolysis temperature increase from 200 to 600˚C (Baldock & Smernik, 2002, Chatterjee *et al.*, 2012, Keiluweit *et al.*, 2010, Schneider *et al.*, 2010, Zimmerman, 2010).

In the last decade, studies on PyOM degradation in soils based on field (Bird *et al.*, 1999, Hammes *et al.*, 2008, Nguyen *et al.*, 2008) and incubation studies (Baldock & Smernik, 2002, Hamer *et al.*, 2004, Hilscher & Knicker, 2011, Kuzyakov *et al.*, 2009, Santos *et al.*, 2012) challenges the view that PyOM persists in soils for millennia (Schmidt & Noack, 2000). A recent synthesis of current knowledge of PyOM degradation in soil estimated turnover times on centennial scales (Singh *et al.*, 2012) . In addition to losses via mineralization, PyOM is also vertically mobile in mineral soil (Brodowski *et al.*, 2007, Dai *et al.*, 2005, Rumpel *et al.*, 2006, Skjemstad *et al.*, 1999) and can eventually be lost from soil by leaching (Abiven *et al.*, 2011, Cheng & Lehmann, 2009, Hockaday *et al.*, 2006, Major *et al.*, 2009b, Shinogi *et al.*, 2003). PyOM dissolution from soils could therefore be an important translocation mechanism in the terrestrial system.

Recent research on soil organic matter (SOM) concludes that long-term persistence of SOM, i.e., on centennial scales, depends on its inaccessibility to microorganisms, such as through organo-mineral interactions (Schmidt *et al.*, 2011, von Lützow *et al.*, 2006). PyOM interacts with the soil mineral phase (Brodowski *et al.*, 2006, Cheng & Lehmann, 2009, Cheng *et al.*, 2006, Glaser *et al.*, 2000) and has been posited to act as a binding

115 agent for soil aggregates (Brodowski *et al.*, 2005a). Vasilyeva et al. (2011) observed that
116 70% of PyOM was associated with the dense fraction (>2 g cm^{-3}) under 55 years of
117 fallow management (fire suppression) in the Streletzkaya steppe (Russia), and suggested
118 its stabilization by clay micro-aggregation. These studies indicate that aggregation and
119 mineral interactions may be important mechanisms for stabilizing PyOM in soil.
120 However, we do not know when and to what extent such stabilizing mechanisms occur
121 during the PyOM decomposition pathway.
122
123 Atmospheric N deposition has increased in recent decades and is predicted to further rise
124 (Galloway *et al.*, 2008). This is important because C and N cycles are closely linked at all
125 scales and interact in many ways and therefore cannot be considered separately (Norby,
126 1998). N addition has decreased (Fog, 1988, Janssens *et al.*, 2010, Turunen *et al.*, 2004,
127 Waldrop *et al.*, 2004), increased (Bragazza *et al.*, 2006, Mack *et al.*, 2004, Waldrop *et al.*,
128 2004), or had no effect on (Knorr *et al.*, 2005) SOM decomposition rates. There are fewer
129 studies on the effects of increased N on wood and PyOM decomposition. These studies
130 observed increased (Allison *et al.*, 2009, Bebber *et al.*, 2011, Micks *et al.*, 2004, Wal *et*
131 *al.*, 2007) or level (McColl & Powers, 1998) decomposition rates for wood, with no
132 effect on PyOM (Santos *et al.*, 2012).
133
134 Recent observations showed that C mineralization rates for native SOM could be
135 influenced from PyOM addition to mineral soils (i.e., priming effect). However, the
136 direction of this priming effect is under debate. PyOM has been shown to inhibit
137 (negative priming) (Jones *et al.*, 2011), have no effect (Abiven & Andreoli, 2010,

Hilscher *et al.*, 2009, Kuzyakov *et al.*, 2009, Santos *et al.*, 2012), or increase native SOM mineralization rates (positive priming) (Steinbeiss *et al.*, 2009, Zimmerman *et al.*, 2011). Moreover, in an incubation study using different types of PyOM, Zimmerman et al. (2011) observed a change in priming effects from positive to negative with time. Several mechanisms have been proposed to explain these contradictory priming effects associated with added PyOM—including, (i) differences in the physico-chemical state of PyOM in different soils (Santos *et al.*, 2012), (ii) encapsulation and/or sorptive protection of SOM by PyOM (Zimmerman *et al.*, 2011), (iii) nutritional competition and balance between r and K strategist microbial population (Fontaine *et al.*, 2003) or (iv) changes in microbial community structure (Blagodatskaya & Kuzyakov, 2008). For wood-amended soil, only a few studies reported positive priming effects on SOM mineralization (Crow *et al.*, 2009, Sulzman *et al.*, 2005) or no priming effect (Santos *et al.*, 2012).

No reported field studies have compared the C and N dynamics of PyOM and its precursor biomass during its decomposition in soil. To evaluate the potential of PyOM as a long-term C sink in soil as compared to its precursor biomass, we conducted a field study to quantify the fate of added $^{13}C/^{15}N$ labeled PyOM and its precursor biomass (*Pinus ponderosa* wood) to a forest soil during ten months, in situ. To determine the effect of added inorganic N on PyOM and wood dynamics in soil, we experimentally manipulated N deposition to half of the plots by adding ammonium nitrate (NH_4NO_3) at (+60 kg N ha^{-1} yr^{-1}). The objectives of our study were to (1) determine recovery of C and N from PyOM and its precursor wood in soil (0–15 cm) after 10 months; (2) quantify the vertical movement of PyOM and wood C and N in the soil profile; (3) investigate the

main stabilization mechanism for PyOM and/or wood by determining the partitioning of PyOM and wood C and N within operational-defined SOM fractions; (4) determine chemical changes in PyOM, both in quality and quantity in comparison to initial input, and (5) assess the effect of N treatment on the decomposition dynamics of PyOM and wood in soil.

2. Materials and Methods

2.1 Field site

The experimental site is a mixed, beech-dominated temperate forest, located 20 km northwest of Zurich, CH (47º 28' 40.8" N, 8º 21' 55.2" E) on the south-facing slope of Lägeren Mountain (eastern-most part of the Jura Mountain range) at 680 m above sea level. The mean annual temperature is 8.4 ºC, and mean annual precipitation is 930 mm (Ruehr & Buchmann, 2010). The soil at the site is classified as a Cambisol (F.A.O.-U.N.E.S.C.O., 1998). Chemical and physical properties of the soil (0–10 cm) are presented in Table 1. Soil volumetric moisture content and soil temperature were monitored every 30 minutes at two depths (5 cm and 10 cm below the surface) within each field replicates, using soil moisture temperature sensors (ECH2O-TE/EC-TM, Decagon Devices, U.S.) connected to a data-logger. Soil moisture in the field site ranged from 20 to 50%, while temperatures ranged from 0ºC to 25ºC (at 5 cm depth) during the first year of the study.

2.2 Experimental design

The experiment is located within a forest gap created by a natural windthrow (in 1999), which has been subsequently mowed to maintain open conditions. The site was chosen to provide similar micro-climatic conditions to a post-fire gap. The experimental setup was completely randomized blocks design, having the factorial combination of three types of organic inputs (i.e. pinewood, PyOM, and no input as control) and two treatment levels of N (ambient =N0 and added N = N+) with three field replicates (n=3) per treatment

207 combination. The ambient, natural N deposition at the field site was estimated to be 20 kg
208 N yr^{-1} ha^{-1} (Kloeti *et al.*, 1989). The N+ treatment corresponds to a level of ca. 80 kg N
209 yr^{-1} ha^{-1} (60 kg N yr^{-1} ha^{-1} added to the ambient N deposition). The wood used for the
210 study was primary stem biomass from two-year-old *Pinus ponderosa* saplings grown
211 under controlled greenhouse conditions and labeled with $^{13}CO_2$ and $^{15}NO_3^-$ (Bird & Torn,
212 2006). PyOM was obtained by charring the labeled wood at 450ºC for 5 hours under N_2
213 flux according to Hammes et al. (2006). The chemical characteristics of both labeled
214 wood (^{13}C = 2.05 atom % and ^{15}N = 4.3 atom %) and PyOM (^{13}C = 2.03 atom % and ^{15}N
215 = 4.2 atom %) are described in Santos et al. (2012). The C and N elemental composition
216 of wood was 499 g kg^{-1} and 4.3 g kg^{-1} respectively. For PyOM, C concentration was 799
217 g kg^{-1} and N was 7.1 g kg^{-1}. The structure of the PyOM and wood are detailed by
218 magnetic resonance, mid-infrared spectroscopy and mass spectrometry in Chatterjee et al.
219 (2012). The wood and PyOM were uniformly labeled (Santos *et al.*, 2012, Yarnes *et al.*,
220 2011). Both wood and PyOM were ground (<2 mm) prior to soil addition.
221
222 In each plot, we inserted 20 cm long and 10 cm diameter mesocosms (polyethylene tubes,
223 smoothed at the top and sharpened at an angle at the bottom) into the soil up to a depth of
224 15 cm from the surface. Each mesocosm had two 4 cm diameter windows (at 7.5 cm and
225 12 cm distance from the bottom and aligned at 120º to one another), fit with 0.7 mm
226 stainless steel mesh to allow fungal hyphae and some fine roots to penetrate the core and
227 limit lateral movement of the added substrate (Bird & Torn, 2006). Mesocosms were
228 placed >1 m from large trees and >0.5 m from the adjacent mesocosms. The mesocosms
229 were installed at the field site in April 2009 and allowed to equilibrate for 180 days

before the addition of the organic inputs.

In October 2009, ^{13}C and ^{15}N enriched-wood and PyOM were applied to the mesocosms at a rate of 189 g C m^{-2} for wood or 397 g C m^{-2} for PyOM, at 2 cm soil depth and mixed gently with 1–2 mm of mineral soil. The PyOM application rate was based on a previous estimate of PyOM inputs to soil after a fire in a similar forest type (Eckmeier *et al.*, 2007b). Unamended-control mesocosms were similarly disturbed to those that received wood or PyOM. Beginning in March 2010, 11.4 mg of $NH_4^+NO_3^-$ dissolved in 10 ml of water was added monthly for 10 months (equivalent to 60 kg N ha^{-1} yr^{-1}) to increased N (N+) treatment mesocosms, while an equivalent amount of distilled water was added to ambient N (N0) treatment mesocosms.

2.3 Soil sampling and analysis

We sampled the intact mesocosms (n=18) 10 months after the PyOM or wood additions to the soil mesocosms. The soil within the mesocosms was separated immediately into 0–5 cm, 5–10 cm and 10–15 cm depth. Soil fauna, stones (>2 mm), and roots (>2 mm) were manually removed and stored separately. Soil subsamples were air-dried and ball-milled for physico-chemical analysis. Soil water content was determined by drying 1 g of soil (n=3) at 105°C for 24 h. Microbial biomass analysis (chloroform fumigation extraction method) was performed immediately after sampling on fresh soil (Vance *et al.*, 1987). Total C and N contents in soil samples were determined with a CHN elemental analyzer (EA 1108 Carlo Erba, Italy). The soil pH values were measured on air-dried soil at mass-to-volume ratio of 1:2.5 (soil:water ratio) (Jackson, 1958).

253

254 **2.4 Soil organic matter fractionation**

255 We used a density fractionation approach to partition SOM into three main pools that
256 differ in their main stabilization mechanisms and turnover times. For each fraction we
257 quantified the ^{13}C and ^{15}N excess from added PyOM or wood to assess the distribution of
258 PyOM and wood into discrete physical fractions as a means for identifying mechanisms
259 such as organo-mineral interactions. In our study, the free light fraction (fLF) was
260 separated using a density of 1.6 g cm^{-3} (Cerli *et al.*, 2012, Glaser *et al.*, 2000) and the
261 occluded light fraction (oLF) was separated after gentle ultrasonic dispersion using a
262 sonifier (Bandelin Sonoplus HD 3400, Germany; calibrated according to Schmidt et al.,
263 (1999a). We applied 250 J ml^{-1} of disruptive energy per sample. This rate was based on
264 analysis of oLF yield and C content across a dispersive energy range (0-300 J ml-1, data
265 not shown). For the density fractionation, a subsample (10 g) of air-dried sample (0-5 cm)
266 was suspended in 50 mL of 1.6 g cm^{-3} sodium polytungstate (SPT) solution (TC-
267 Tungsten compounds), the suspension was allowed to settle for 1 h and centrifuged (3237
268 g, 30 minutes; Heraeus Megafuge 1.0, UK). The floating material ($\leq$ 1.6 g cm^{-3}) was
269 collected as fLF on a glass microfiber filters with 1.5 µm particle retention (934-AH,
270 Whatman), washed thoroughly with deionized water to remove any SPT (conductivity of
271 supernatant water <50 µS cm^{-1}) and freeze-dried. The remaining pellet for each sample
272 was re-suspended in SPT and treated with ultra sonification (250 J ml^{-1}) to destroy
273 aggregates. The suspension was allowed to settle for 4 h, followed by centrifugation
274 (3237 g, 30 minutes). The oLF (< 1.6 g cm^{-3}) was collected similarly as above on glass
275 microfiber filters and washed thoroughly with deionized water (conductivity of

supernatant water <50 μS cm^{-1}). The remaining dense fraction (DF) was washed until the SPT was removed completely (conductivity of supernatant water <50 μS cm^{-1}). It should be noted that DF was not further separated via physical fractionation into sand or clay/silt fractions. Therefore, DF is heterogeneous and the presence of organic matter in DF would not necessarily mean complete organo-mineral interactions. Some part of organic matter present in DF could also be uncomplexed organic matter in sand sized separates. The density fractions (fLF, oLF, DF) were ball-milled to homogenize the samples. C and N concentration was measured with an elemental analyzer (EA 1108 Carlo Erba, Italy) and ^{13}C and ^{15}N was measured using an isotope ratio mass spectrometer (IRMS) (Delta S, Thermo Finnigan, U.S.). The recovery of C and N was calculated based on the amount of C and N present in 0-5 cm depth soil after 10 months.

2.5 Benzenepolycarboxylic acid (BPCA) analysis

The BPCA molecular marker method was employed to quantify and characterize the PyOM before and after its addition to the soil (Brodowski *et al.*, 2005b, Glaser *et al.*, 1998, Schneider *et al.*, 2010). Subsamples (400–500 mg) of PyOM-amended air-dried soil (0–5 cm, n=3) and PyOM (30–40 mg) were pre-treated with 4M trifluroacetic acid (4 h, 105°C) to remove Fe and Al, followed by conversion of PyOM into BPCAs by nitric acid oxidation (8 h, 170°C). The digested extract was further purified using cation-exchange resin to remove any polyvalent cations. The extracts were freeze-dried and subsequently derivatized into trimethylsilyl derivatives to be analyzed on a gas chromatograph (Agilent 6890) equipped with a flame ionization detector and a DB-5MS capillary column (50 m × 0.2 mm i.d., 0.33 μm film thickness). Each analysis was

299 performed in triplicate. The acids with 3, 4, 5, and 6 carboxyl functions (B3CA, B4CA,
300 B5CA, and B6CA, respectively) were identified and summed up to represent the total
301 amount of pyrogenic molecular markers in the PyOM.
302
303 **2.6 Microbial biomass by chloroform fumigation direct extraction (CFDE)**
304 Moist soil, equivalent of 20 g of oven-dried soil (105ºC, 24 h), was fumigated with
305 alcohol free chloroform in desiccators for 24 hours in the dark (Vance *et al.*, 1987). The
306 fumigated soil and an equivalent amount of non-fumigated soil for each sample was then
307 extracted using 1M KCl (1:5 soil solution ratio) for 1 hour, filtered (Whatman GF 934-
308 AH), and extracts stored at -20 ºC until analysis. The total organic C (TOC) in fumigated
309 and non-fumigated extracts were analyzed using a TOC analyzer (TOC-V, Shimadzu
310 Corporation, Japan). A conversion factor of 0.45 (K_c) (Wu *et al.*, 1990) and 0.68 (K_n)
311 (Shen *et al.*, 1984) was applied for incomplete extraction for microbial C and N,
312 respectively. It should be noted that PyOM could reabsorbs lysed cells and may influence
313 microbial biomass recovery (Durenkamp *et al.*, 2010, Liang *et al.*, 2010). Nevertheless,
314 the amount of PyOM C contributing to total SOC is inversely correlated to the extraction
315 efficiency (Liang *et al.*, 2010). In this study, the applied PyOM C contributed to 11% of
316 total SOC and therefore, reabsorption of lysed microbial biomass on PyOM is assumed to
317 be negligible. The $\delta^{13}C$ and $\delta^{15}N$ of extracts were analyzed on freeze-dried extracts using
318 IRMS (Delta S, Thermo Finnigan) (Murage & Voroney, 2007)
319 The $\delta^{13}C$ (‰) of soil microbial biomass C (SMB-C) was estimated as the $\delta^{13}C$ of the C
320 extracted from the fumigated sample in excess of that extracted from the non-fumigated
321 sample, using Eq. 1 (Murage & Voroney, 2007),

322

$$\delta^{13}C = \frac{(\delta^{13}C_f \times C_f) - (\delta^{13}C_{nf} \times C_{nf})}{(C_f - C_{nf})} \quad (1)$$

323

324 where C_f and C_{nf} were the amounts of C extracted from the fumigated and non-fumigated
325 samples (μg C g^{-1} dry soil) and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ were the ^{13}C natural abundance of the
326 fumigated and non-fumigated extracts (‰), respectively.

327

328 The soil under no treatment (control plots) was taken as the reference, and the proportion
329 of labeled substrate-derived-C in SMB was calculated using Eq. 2,

330

$$f_{C-substrate}, \% = \left(\frac{\delta_{treated-microbes} - \delta_{control\ microbes}}{\delta_{substrate} - \delta_{control\ microbes}}\right) \times 100 \quad (2)$$

331

332 where $\delta_{treated\text{-}microbes} = \delta^{13}C$ value of SMB extracted from the substrate-treated mesocosms,
333 $\delta_{substrate}$ = 842‰ for wood and 800‰ for PyOM, and $\delta_{control\ microbes} = \delta^{13}C$ the value of
334 SMB extracted from the control mesocosms.

335

336 **2.7 Calculations**

337 The amount of labeled substrate-C (or N) (PyOM and wood) recovered in the bulk soil
338 and density fractions was calculated for each mesocosms using a two end-member linear
339 mixing model (Eq. 3 and Eq. 4) (Bernoux *et al.*, 1998),

$$f_{substrate} = \frac{\delta_{soil\ sample} - \delta_{control\ soil}}{\delta_{substrate} - \delta_{control\ soil}} \quad (3)$$

340

$$Amount\ of\ substrate_{C\ or\ N}, \% = C\ or\ N_{sample}(\%) \times f_{substrate} \quad (4)$$

341

342 where $f_{substrate}$ is the fraction of substrate in the soil, $\delta_{soil\ sample}$, $\delta_{control\ soil}$, $\delta_{substrate}$ is
343 isotopic value (either $\delta^{13}C$ or $\delta^{14}N$, ‰) of soil sample, corresponding control soil within
344 field replicates, and substrate (PyOM or wood), respectively. *C (or N)* is the amount of C
345 (or N) in % of the soil sample.

346

347 To calculate excess ^{13}C or ^{15}N values in soil and microbial biomass, we used Eq. 5
348 (Dawson *et al.*, 2002),

$$Excess\ ^{13}C\ or\ ^{15}N = \frac{atom\%_{sample} - atom\%_{background}}{100} \times C\ or\ N \quad (5)$$

349

350 where *Excess ^{13}C or ^{15}N* (μg g^{-1} dry soil for microbes) is the total amount of ^{13}C or ^{15}N
351 added by labeled PyOM or wood to soil or microbial biomass, $atom\%_{sample}$ is the atom %
352 of soil or microbial biomass in substrate treated sample, and $atom\%_{background}$ is the atom%
353 of soil or microbial biomass in the control treatments averaged over 3 plots. *C* or *N* is the
354 total organic C or N content of soil (g kg^{-1} soil) or microbial biomass (μg g^{-1} dry soil).

355

356 To estimate the potential migration rate of substrate-C in the soil profile, we use Eq. 6

$$Migration\ rate_{input-C} = \frac{d_{max}}{t} \quad (6)$$

357

358 where d_{max} (mm) is the maximum depth where PyOM was recovered below its
359 application depth after time t (in years). We estimated d_{max} as 10.5 cm based on the
360 recoveries of PyOM and wood at depth 10-15 cm (average of 12.5 cm as soil was
361 homogenized) and application depth at 2 cm below the surface.

362

363 **2.8 Statistical analysis**

364 We performed an analysis of variance (ANOVA) using SPSS (IBM-SPSS statistics 20.0
365 package for Mac) to determine the effect of the different input treatments and the two
366 levels of N application, and the interactions between the two factors at different depths
367 (0-5 cm, 5-10 cm and 10-15 cm). Differences in the relative contribution of individual
368 molecular markers and BPCA-C (g kg^{-1} OC) at t=0 and t= 10 months were tested with
369 independent t test, when the data were normally distributed (Shapiro-Wilk test), and non-
370 parametric Mann-Whitney U-test when the normality test failed. Levene's test for
371 equality of variance was used to determine homogeneity in the data, and significance test
372 was used accordingly. We considered differences between treatments with $p \leq 0.05$ as
373 significant.

374

375 **3. Results**

376

377 **3.1 Total C and N and recovery of substrate-C or N in the topsoil**

We observed no change in soil total C in mesocosms (0-15 cm) due to different organic inputs or N ($p>0.05$, $n=3$), except in wood-amended soil under the N+ treatment which had a significantly higher total C than N0 at 0-5 cm depth ($p = 0.007$). Including all depth and across all N treatments, we recovered 99.6% ± 0.2% of the initial ^{13}C-PyOM and 48.3% ± 4.4% of the initial ^{13}C-wood after 10 months (Table 2). Most of the ^{13}C-PyOM (95% ± 1.7% for N0 and 96.5% ±1.4% for N+) and ^{13}C-wood (40.0% ± 13.2% under N0 and 44.5% ± 1.4% under N+) were recovered between 0–5 cm. Both PyOM-C and wood-C were recovered until the depth 10-15 cm indicating vertical movement with a migration rate of 126 mm yr^{-1} and 3-4% of PyOM-C and 4-8% of wood-C migrated below the incorporation depth of 2 cm (Table 2). It should be noted that the migration rate of PyOM and wood C could range between 100 mm y^{-1} to 150 mm y^{-1}as only three depth separation was chosen in this study. We observed no N effect either on the vertical movement (amount or rates) or C recovery for both wood and PyOM.

Similarly, total soil N in mesocosms was not affected by added PyOM, wood or from added N. We recovered >90% of applied ^{15}N-PyOM, mainly at the application depth (0–5 cm; Table 2). The recovery of ^{15}N-PyOM across all N treatment (93.6% ± 2.2%, $n=6$) in mesocosms was significantly lower than ^{13}C-PyOM ($p\leq0.05$). More wood-^{15}N was recovered in mesocosms under ambient N levels (88.5% ± 6.4%) than with elevated (N+) levels (48.0% ± 6.0%, Table 2).

3.2 Soil organic matter fractions

The average recovery of C and N of bulk soil across all treatments (n = 18) after density fractionation was 88.8% ± 2.9% of total C and 84.8% ± 2.6% of the total N, respectively (Table 3). The loss of C and N is due to mobilization into SPT solution or as dissolved organic C and N during washing of density fractions with water to remove SPT, which were discarded during the process. After density fractionation, we recovered on average all of ^{13}C-PyOM (100% ± 3.7%, n = 6) and ^{15}N-PyOM (100% ± 6%, n = 6), but recovery of ^{13}C-wood (72.0% ± 9.7%; n = 6) and ^{15}N-wood (70% ± 14%; n = 6) was highly variable. The recovery of ^{13}C-wood was significantly lower than ^{13}C-PyOM ($p \leq 0.05$), while ^{15}N-wood recovery was highly variable among replicates, leading to no significant difference in ^{15}N-PyOM recoveries. It should be noted that the recoveries of PyOM and wood C and N are expressed as % of the amount recovered in the bulk soil between 0-5 cm depth after 10 months.

For all treatments, most of the SOC was in the DF. The C concentration and C:N ratios of density fractions increased in the order DF< fLF <oLF (Table 4). Relative to the unamended control, the C:N ratio of both PyOM and wood-amended soil was significantly higher ($p \leq 0.05$) in fLF and oLF density fractions , while DF had similar values to the control soils across all treatments. The important contribution of PyOM (22% of total C in fLF and 7% of total C in oLF was PyOM C) and wood (5% of total C in fLF and 3% of total C in oLF-C was wood C) in these fractions explains their higher C:N ratio. We did not observe any significant effects of N treatment on the C:N ratios in density fractions across treatment.

423 The distribution of PyOM-C and wood-C among the density fractions after 10 months of
424 their application was not similar (Fig. 1). Under ambient N, PyOM-C was recovered on
425 average (n =3) mostly in the fLF (70.2% ± 4.8%) followed by oLF (22.7% ± 3.9%) while
426 wood C was recovered mostly in oLF (42.6% ± 21.5%) and fLF (39.4% ± 20.8%). The
427 DF showed least recovery for both organic-input C under ambient N but wood C (14.6%
428 ± 9.4%) recovery in DF was double as compared to PyOM C (7.1% ± 1.2%). A similar
429 trend was observed in the increased N treatment for PyOM C with 77.4% ± 2.2% in fLF,
430 17.3% ± 1.9% in oLF and 5.4% ± 0.9%. Wood C under increased N treatment, however,
431 showed maximum recovery in the DF (38.6% ± 5.4%) followed by fLF (38.0% ± 11.5%)
432 and oLF (23.3% ± 9.3%). Added N resulted in significantly higher wood C recovery in
433 DF as compared to wood C recovery under ambient N ($F = 9.6$, $p = 0.02$) and PyOM C
434 recovery under increased N treatment ($F=18.3$, $p = 0.003$).
435
436 In PyOM-amended soils, across all N treatments (n=6) we observed a decrease of 13% ±
437 3% in fLF, 4% ± 3% in oLF and 0.2% ± 0.3% in DF in native soil C concentration
438 (priming effect - Figure 2). Native soil C concentration in wood-amended soils did not
439 show a significant change in fLF (4.5 ± 4.6%, $p = 0.061$) or oLF (3.3 ± 3.5%, $p = 0.068$).
440 Under the added N treatment, wood-amended soil showed a significant increase in the
441 DF-C (0.7±0.3%, $p=0.04$) and bulk soil (1.3±0.3 %, $p=0.01$) with respect to zero. Added
442 N did not affect native SOC content or interact with PyOM effects on SOC content (Fig.
443 2). Native soil organic N showed little modification in either wood or PyOM-amended
444 soil.
445

446 **3.3 PyOM quality and quantity using BPCA marker molecules**

447 The BPCA-C content of the labeled PyOM used in this study was 145.9 ± 6.8 g BPCA-
448 C/kg OC (without using any conversion factor) and is similar to standard PyOM
449 produced at 450°C (Schneider *et al.*, 2010). The BPCA-C content of the labeled PyOM
450 mixed with soil (1:137 PyOM: dry soil mass ratio that corresponds to the application rate
451 at 0–5 cm depth) at time t = 0 was 28.7 ± 2.6 g BPCA-C/kg OC. Ten months after PyOM
452 addition to soils, total BPCA-C content in the 0-5 cm depth was not significantly different
453 than at t=0 (28.5± 2.6 g BPCA-C kg^{-1} OC for N0 and 23.7±14 g BPCA-C kg^{-1} OC for
454 N+, $p>0.05$).
455
456 We observed a significant increase in benzene tetracarboxylic acids (B4CA, $p = 0.004$)
457 and a significant decrease in benzene hexa-carboxylic acids (B6CA, $p = 0.023$), and
458 therefore a shift in the relative contribution of individual molecular markers after 10
459 months (Fig. 3). We did not observe any significant change in the relative proportion of
460 either benzene tricarboxylic acids (B3CA) or benzene penta-carboxylic acid (B5CA) and
461 no significant effect of added N on BPCA distribution patterns.
462
463 **3.4 Microbial C and N**
464 Microbial biomass C (μg per g dry soil) was unaffected by addition of wood or PyOM
465 after ten months in the mesocosms (Table 4). Microbial biomass C declined significantly
466 with increasing depths (0–5, 5–10 and 10–15 cm) averaged across all treatments
467 ($p<0.05$). ^{13}C-wood contributed 6–10% of microbial biomass, two levels of magnitude
468 higher than from ^{13}C-PyOM (between 0.14–0.18 % PyOM-^{13}C).

469

470 Microbial biomass N (μg per g dry soil) decreased with increasing depth from 0-5 cm to
471 10 cm to 15 cm (Table 4), except for wood-amended soil under N0 treatment. In the 0–5
472 cm depth, microbial biomass N increased ($p = 0.09$) with added N in wood-amended soil
473 (51 ± 17 μg N g^{-1} dry soil under N0 and 89 ± 5 μg N g^{-1} dry soil under N+), while PyOM
474 showed an opposite trend (94 ± 51 μg N g^{-1} dry soil under N0 and 64 ± 30 μg N g^{-1} dry
475 soil under N+) but it was not significant (Table 5). Despite the highly labeled material
476 used in this study, the ^{15}N signal was not detectable from added PyOM.

477

478 **4. Discussion**

479

480 **4.1 Loss of PyOM and wood by decomposition and downward migration in soil**

481 We recovered >99% PyOM-C but only half of wood-C in the soil mesocosms after 10
482 months in situ averaged across all treatments, which is consistent with the results from
483 the same site for wood (Kammer & Hagedorn, 2011). The loss of pine wood C
484 corresponds to annual to decadal turnover times, which is similar to that estimated by a
485 180 days laboratory incubation of the same wood in two Alfisols of different parents
486 material (Santos *et al.*, 2012) and by a 1 year litter experiment using ^{13}C labeled twigs
487 (Beech tree, 1-8 mm) in the same field study area (Kammer & Hagedorn, 2011).

488

489 In the present field study, the mean difference of PyOM stocks over 10 months was
490 approximately 1% of the amount added, but this difference was not significant so we did
491 not estimate a turnover time of PyOM. Nevertheless, we can conclude that PyOM

492 decomposed much slower than the plant biomass from which it was derived after
493 pyrolysis. Several field and laboratory studies have found PyOM turnover times on
494 centennial scale (Singh *et al.*, 2012). Our results provide in situ confirmation of the
495 relative rates reported by previous laboratory studies. (Baldock & Smernik, 2002,
496 Hilscher & Knicker, 2011, Santos *et al.*, 2012).
497
498 We recovered 3-4% of applied PyOM below the application depth (0-5 cm) suggesting
499 downward vertical movement of PyOM. Leifeld *et al*., (2007) estimated migration rate of
500 630-1160 mm y^{-1} in a peat soil with very low bulk density where 21-69% migrated below
501 the incorporation depth in 95 years. Compared to the previously cited study, the soil here
502 was denser, more clayey and so probably less favorable to organic matter transfer through
503 the profile. Nevertheless, the amount of translocated PyOM to lower depth in the present
504 case was higher than Major *et al*., (2009a) who observed 100 mm y^{-1} migration rate in a
505 sandy Oxisol under native savanna vegetation with only 0.45% of applied amount.
506 Therefore, translocation of PyOM to lower depth in the present case could either be due
507 to soil faunal mixing (Carcaillet, 2001, Eckmeier *et al.*, 2007a, Topoliantz & Ponge,
508 2003) or leaching. The downward vertical movement could either result in its
509 accumulation at lower depths (Brodowski *et al.*, 2007, Dai *et al.*, 2005, Rumpel *et al.*,
510 2006, Skjemstad *et al.*, 1999) or loss from soil as DOC (Ding *et al.*, 2013, Dittmar *et al.*,
511 2012, Hockaday *et al.*, 2007). On the other hand, we observed that wood C translocated
512 to lower depths is double (4-8%) in comparison to PyOM C. Transport of wood-C into
513 deeper soil horizons usually occurs as dissolved organic carbon (DOC) (Yano *et al.*,
514 2005, Zalamea *et al.*, 2007). This could partly explain the larger amount of wood being

translocated to deeper soil profile.

4.2 Quality of PyOM changes within one year in soil

After 10 months in soil, the total BPCA-C content of PyOM was unchanged, but the proportion of various BPCAs had changed. Aromatic condensation, measured as the relative contribution of B6CA to the total BPCA-C (Brodowski, 2005, Schneider *et al.*, 2010), decreased after 10 months, suggesting that PyOM partially degraded into smaller aromatic moieties. In contrast, Hammes et al. (2008), in a field study, observed a relative increase in the B6CA molecular marker after 100 years *in situ* (no absolute change) suggesting relative preservation of condensed aromatic structures. Moreover, Schneider et al. (2011) found no change in the BPCAs pattern in a 100-year chronosequence. It is not clear yet if the changes in the relative contribution of various BPCAs could be linked directly to decomposition mechanism. Abiven *et al*. (2011) observed an increase in the B3CA at the expense of B5CA between a fresh and a 10 y aged PyOM while Brodowski (2005) also observed similar results in an incubation study on the decomposition of PyOM. The dominance of B3CA and B4CA indicates small aromatic cluster size (Schneider, 2011) and indicates depolymerization of the highly condensed aromatic backbone of PyOM (Kaal *et al.*, 2009). These previous findings, together with our data, suggest that PyOM in soil degrades by the breaking of condensed aromatic structures into smaller clusters, at least within the first stages of degradation after the input of PyOM to the soil.

4.3 PyOM was physically associated with the soil mineral fraction after 10 months

538 About one-third of the applied PyOM C was recovered in aggregates (i.e. oLF) plus
539 dense fraction of soil within one year. Studies of the ambient distribution of PyOM in soil
540 fractions, in other words, in soils collected from sites without intentional addition of
541 PyOM also find significant portion of PyOM in these fractions (Brodowski *et al.*, 2006,
542 Glaser *et al.*, 2000, Laird *et al.*, 2008, Liang *et al.*, 2008, Vasilyeva *et al.*, 2011).
543 However, these studies do not report the temporal scale over which aggregation and/or
544 organo-mineral interaction of PyOM with soil occurs, as they have no data for PyOM
545 inputs to these soils. The present study highlights, for the first time, that significant
546 interaction between PyOM and the mineral phase of soil can occur *in situ* within a year.
547 Glaser et al. (2000) posit that interaction of PyOM and soil mineral phases might stabilize
548 PyOM by aggregation and organo-mineral associations. As one mechanism, oxidation of
549 PyOM surface has been hypothesized to favor its interaction with the soil mineral phase
550 (Brodowski *et al.*, 2006). It was, however, not possible to directly link oxidized forms of
551 PyOM to a specific interaction with soil minerals that led to recovery of PyOM in the
552 dense fraction. Moreover, the presence of PyOM in DF does not mean that all of it was
553 mineral stabilized and PyOM could also occur as uncomplexed sand fraction.
554

555 **4.4 PyOM accelerated the loss of native C from free light fraction (fLF)**

556 We observed positive priming in the free particulate native C pool in soil (i.e. in the fLF)
557 by both PyOM and wood, with significantly larger priming by PyOM than wood of native
558 SOC in the fLF. However, there is no consistent effect of PyOM on native SOC across
559 the literature. For example, Santos *et al*. (2012) did not observe priming in SOM using
560 the same substrates but different soils in an incubation study, which indicated that the

type of soil has an influence on priming effects rather than the organic substrate itself. Moreover, Stewart et al., (2011) observed exponential relationship between initial SOC and cumulative soil C primed by PyOM addition with high negative priming at low soil C% and a positive priming at high soil C%. Our study, for the first time, indicated the pool of SOM that is affected due to priming by organic input. The native SOM associated to the minerals is not affected by the input while the SOM that is free or occluded in aggregates decreased significantly within few months. Therefore, we conclude that soils containing a large fraction of free SOM are probably more vulnerable to priming effect.

One property supporting priming is PyOM's porous structure which is known to sorb organic substrates (Accardi-Dey & Gschwend, 2002, Chen *et al.*, 2008, Kwon & Pignatello, 2005, Raveendran & Ganesh, 1998, Sudhakar & Dikshit, 1999) and may offer favorable microsites for microorganisms and shelter them against soil faunal predators (Pietikäinen *et al.*, 2000). If so, the physical effects of PyOM amendments could increase microbial activity and lead to increased mineralization of readily decomposable substrates such as the fLF. However, we were not able to detect a change in microbial biomass (see next section), and further research is needed to develop a predictive understanding of the temporal course of priming-type effects.

4.5 No change in microbial biomass

PyOM addition had no effect on SMB-C 10 months after addition to the soil mesocosm. Bruun et al. (2008) observed a similar lack of effect in soil treated with PyOM (^{14}C-labeled roots of barley). On the contrary, Steinbeiss et al. (2009) observed a reduction in

584 microbial biomass in soil to which charred glucose had been added to a forest soil in an
585 incubation study after 4 months. Several studies observed increased microbial biomass
586 and activity in soil (Bruun *et al.*, 2011, Kolb *et al.*, 2009, Steiner *et al.*, 2008) within days
587 to few months after PyOM addition or higher SMB-C in PyOM rich soils compared to
588 control or adjacent soils with no PyOM (Liang *et al.*, 2010). Our study was comparatively
589 longer than the studies cited above (10 months versus a couple of weeks) and therefore
590 we cannot exclude the possibility that the microbial biomass could have increased in the
591 first few weeks after PyOM addition and reverted to its initial value over time. Moreover,
592 The present study is the case of a single input of PyOM to the soil without a real wildfire
593 and therefore its effect on the soil properties and consequently on microbial biomass is
594 not similar to PyOM rich soils. In addition, the inconsistency in the response of microbial
595 biomass to PyOM treatment in soil may be attributed to type of PyOM used that differed
596 in the degree of condensation, intensity of pyrolysis and amount of pre-combustion
597 material present in various studies (Zavalloni *et al.*, 2011). PyOM could have influenced
598 the extraction efficiency but in our study it would have not resulted in a major change, as
599 the amount of PyOM used was small as compared to total SOC. Our results suggest that,
600 under these conditions, the total microbial biomass is not durably affected by the
601 substrate addition
602
603 We observed a small amount of PyOM-C within the microbial biomass 10 months after
604 organic inputs to soil, as did Brunn et al. (2008) and Zavalloni et al. (2011). Kuzyakov et
605 al. (2009) observed a higher assimilation of PyOM (1.5–2.6% of initial PyOM input) by
606 microbial biomass after 624 days of incubation compared to our 10 months field study. In

607 all these studies, the amount of PyOM incorporated into the SMB was large enough to be
608 detected clearly, and hence indicate that microbes can utilize PyOM as a C source.
609
610 **4.6 Effect of N fertilization**
611 The addition of N had little impact on the parameters considered in this study. Under the
612 added N treatment, we observed a significant increase in the total C content at 0-5 cm
613 depth in wood amended soil, higher loss of wood-N, a higher transfer of wood derived C
614 in the DF and a higher N content in the microbial biomass. However, these changes are
615 limited and affect the C and N cycles only marginally. These results are in line with
616 Santos et al., (2012). However, the effect of N addition is often seen in the longer term
617 and these results need to be validated for longer periods.
618
619 **4.7. Summary**
620 The novel use of a dual isotope label for PyOM and wood in this field study provided
621 insight into the dynamics of PyOM and wood C and N during the first year after its
622 application to soil. The PyOM C was almost completely retained in the soil, although
623 there was a small but significant change in the overall chemical structure of PyOM. In
624 contrast, 48% of the initial amount of wood C was lost. Our results showed that PyOM
625 persistence in soil need not solely be due to its chemical structure, as one-third of the
626 PyOM C was quickly incorporated into physically protected fractions (i.e. oLF and DF).
627 PyOM primed the loss of native soil C in the free light fraction, suggesting a significant
628 loss of decadal C pool of SOM.
629

630 The results of this study recognize the suitability of PyOM to sequester C in soils. C and
631 N losses from PyOM were negligible after 10 months and were hardly utilized by
632 microbes. Moreover, PyOM interacted with soil mineral phase within 10 months which
633 would result in further C stabilization in the soil. In this context, the soil physical
634 properties need to be considered. Nevertheless, the stabilization of C in soil via PyOM
635 amendment could be offset by increased priming in the labile soil organic pool. Also, in
636 the long term, it is important to take into account the losses due to mobilization and
637 transport of PyOM C and N in the deeper soil profile. Our study demonstrated that PyOM
638 underwent modification at the molecular scale with breakdown into smaller aromatic
639 moieties within 10 months. This can also have implication on the stability of PyOM on
640 the long-term. Furthermore, the dynamics of PyOM was not affected significantly due to
641 N addition. The dynamics of PyOM in soil is complex with degradation, translocation
642 and stabilization occurring parallel to each other and needs further mechanistic
643 investigation on the long term.
644
645
646
647 **Acknowledgements**
648 The Swiss National Science Foundation (SNSF) financially supported this study. This
649 work was also supported by the Director, Office of Science, Office of Biological and
650 Environmental Research, Climate and Environmental Science Division, of the U.S.
651 Department of Energy under Contract No. DE-AC02-05CH11231 to Berkeley Lab. This
652 study is part of university of Zurich Research Priority Program “Global Change

653 and Biodiversity". We would like to thank Sarah Bösch and Ryan Christinger for their
654 help in setting the experimental field plots, and Ivan Woodhatch for the technical help
655 and support in the setup. We also thank Michael Hilf, Bruno Kägi, and Claudia Schreiner
656 for their assistance in various laboratory analyses carried at the University of Zurich. We
657 further extend our thanks to Alois Zürcher for support in TOC analysis at the Institute for
658 Forest, Snow and Landscape research (WSL). We would also like to acknowledge Dr.
659 Rolf Siegwolf, Dr. Matthias Saurer, and Catharina Lötscher for support in the isotope
660 analysis at Paul Scherrer Institute (PSI), Switzerland. We also extend our thanks to Dan
661 Hawkes, Berkeley Labs for editing and proofreading this manuscript.

662

663

664

665

666

667

668

669

670

671 ***References***

672

673 Abbott DT, Crossley DA (1982) Woody litter decomposition following clear-cutting.
674 *Ecology,* **63**, 35-42.
675 Abiven S, Andreoli R (2010) Charcoal does not change the decomposition rate of mixed
676 litters in a mineral cambisol: a controlled conditions study. *Biology and Fertility*
677 *of Soils,* **47**, 111-114.
678 Abiven S, Hengartner P, Schneider MPW, Singh N, Schmidt MWI (2011) Pyrogenic
679 carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh
680 charcoal. *Soil Biology & Biochemistry,* **43**, 1615-1617.

681 Accardi-Dey A, Gschwend PM (2002) Assessing the combined roles of natural organic
682 matter and black carbon as sorbents in sediments. *Environmental Science &*
683 *Technology,* **36**, 21-29.
684 Allison SD, Lebauer DS, Ofrecio MR, Reyes R, Ta AM, Tran TM (2009) Low levels of
685 nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology &*
686 *Biochemistry,* **41**, 293-302.
687 Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally,
688 altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry,* **33**, 1093-1109.
689 Bebber DP, Watkinson SC, Boddy L, Darrah PR (2011) Simulated nitrogen deposition
690 affects wood decomposition by cord-forming fungi. *Oecologia,* **167**, 1177-1184.
691 Bernoux M, Cerri CC, Neill C, De Moraes JFL (1998) The use of stable carbon isotopes
692 for estimating soil organic matter turnover rates. *Geoderma,* **82**, 43-58.
693 Bird JA, Torn MS (2006) Fine roots vs. Needles: A comparison of C-13 and N-15
694 dynamics in a ponderosa pine forest soil. *Biogeochemistry,* **79**, 361-382.
695 Bird MI, Moyo C, Veenendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon
696 in a savanna soil. *Global Biogeochemical Cycles,* **13**, 923-932.
697 Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects
698 and their dependence on soil microbial biomass and community structure: critical
699 review. *Biology and Fertility of Soils,* **45**, 115-131.
700 Bragazza L, Freeman C, Jones T *et al.* (2006) Atmospheric nitrogen deposition promotes
701 carbon loss from peat bogs. *Proceedings of the National Academy of Sciences,*
702 **103**, 19386-19389.
703 Brodowski S (2005) Origin, function, and reactivity of black carbon in the arable soil
704 environment. *PhD Thesis, Institut für Bodenkunde, Bonn*, 183.
705 Brodowski S, Amelung W, Haumaier L, Abetz C, Zech W (2005a) Morphological and
706 chemical properties of black carbon in physical soil fractions as revealed by
707 scanning electron microscopy and energy-dispersive X-ray spectroscopy.
708 *Geoderma,* **128**, 116-129.
709 Brodowski S, Amelung W, Haumaier L, Zech W (2007) Black carbon contribution to
710 stable humus in German arable soils. *Geoderma,* **139**, 220-228.
711 Brodowski S, John B, Flessa H, Amelung W (2006) Aggregate-occluded black carbon in
712 soil. *European Journal of Soil Science,* **57**, 539-546.
713 Brodowski S, Rodionov A, Haumaier L, Glaser B, Amelung W (2005b) Revised black
714 carbon assessment using benzene polycarboxylic acids. *Organic Geochemistry,*
715 **36**, 1299-1310.
716 Bruun EW, Muller-Stover D, Ambus P, Hauggaard-Nielsen H (2011) Application of
717 biochar to soil and N2O emissions: potential effects of blending fast-pyrolysis
718 biochar with anaerobically digested slurry. *European Journal of Soil Science,* **62**,
719 581-589.
720 Bruun S, Jensen ES, Jensen LS (2008) Microbial mineralization and assimilation of black
721 carbon: Dependency on degree of thermal alteration. *Organic Geochemistry,* **39**,
722 839-845.
723 Busse MD (1994) Downed bole-wood decomposition in lodgepole pine forests of central
724 Oregon. *Soil Science Society of America Journal,* **58**, 221-227.

725 Carcaillet C (2001) Are Holocene wood-charcoal fragments stratified in alpine and
726 subalpine soils? Evidence from the Alps based on AMS C-14 dates. *Holocene,* **11**,
727 231-242.
728 Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and
729 heavy organic matter fractions in soil - Testing for proper density cut-off and
730 dispersion level. *Geoderma,* **170**, 403-416.
731 Chambers JQ, Schimel JP, Nobre AD (2001) Respiration from coarse wood litter in
732 central Amazon forests. *Biogeochemistry,* **52**, 115-131.
733 Chatterjee S, Santos F, Abiven S, Itin B, Stark RE, Bird JA (2012) Elucidating the
734 chemical structure of pyrogenic organic matter by combining magnetic resonance,
735 mid-infrared spectroscopy and mass spectrometry. *Organic Geochemistry,* **51**, 34-
736 44.
737 Chen BL, Zhou DD, Zhu LZ (2008) Transitional adsorption and partition of nonpolar and
738 polar aromatic contaminants by biochars of pine needles with different pyrolytic
739 temperatures. *Environmental Science & Technology,* **42**, 5137-5143.
740 Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient.
741 *Chemosphere,* **75**, 1021-1027.
742 Cheng CH, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black
743 carbon by biotic and abiotic processes. *Organic Geochemistry,* **37**, 1477-1488.
744 Clark DB, Clark DA, Brown S, Oberbauer SF, Veldkamp E (2002) Stocks and flows of
745 coarse woody debris across a tropical rain forest nutrient and topography gradient.
746 *Forest Ecology and Management,* **164**, 237-248.
747 Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA (2009) Sources
748 of plant-derived carbon and stability of organic matter in soil: implications for
749 global change. *Global Change Biology,* **15**, 2003-2019.
750 Dai X, Boutton TW, Glaser B, Ansley RJ, Zech W (2005) Black carbon in a temperate
751 mixed-grass savanna. *Soil Biology & Biochemistry,* **37**, 1879-1881.
752 Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in
753 plant ecology. *Annual Review of Ecology and Systematics,* **33**, 507-559.
754 Deluca TH, Aplet GH (2008) Charcoal and carbon storage in forest soils of the Rocky
755 Mountain West. *Frontiers in Ecology and the Environment,* **6**, 18-24.
756 Ding Y, Yamashita Y, Dodds WK, Jaffe R (2013) Dissolved black carbon in grassland
757 streams: Is there an effect of recent fire history? *Chemosphere,* **90**, 2557-2562.
758 Dittmar T, Paeng J, Gihring TM, Suryaputra IGNA, Huettel M (2012) Discharge of
759 dissolved black carbon from a fire-affected intertidal system. *Limnology and*
760 *Oceanography,* **57**, 1171-1181.
761 Durenkamp M, Luo Y, Brookes PC (2010) Impact of black carbon addition to soil on the
762 determination of soil microbial biomass by fumigation extraction. *Soil Biology &*
763 *Biochemistry,* **42**, 2026-2029.
764 Eckmeier E, Gerlach R, Skjemstad JO, Ehrmann O, Schmidt MWI (2007a) Minor
765 changes in soil organic carbon and charcoal concentrations detected in a
766 temperate deciduous forest a year after an experimental slash-and-burn.
767 *Biogeosciences,* **4**, 377-383.
768 Eckmeier E, Rosch M, Ehrmann O, Schmidt MWI, Schier W, Gerlach R (2007b)
769 Conversion of biomass to charcoal and the carbon mass balance from a slash-and-
770 burn experiment in a temperate deciduous forest. *Holocene,* **17**, 539-542.

771 F.A.O.-U.N.E.S.C.O. (1998) ISSS, ISRIC, FAO. World Reference Base for Soil
772 Resources. FAO, World Soil Resources Reports 84. pp Page, Rome.
773 Fasth BG, Harmon ME, Sexton J, White P (2011) Decomposition of fine woody debris in
774 a deciduous forest in North Carolina. *Journal of the Torrey Botanical Society,*
775 **138**, 192-206.
776 Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic-
777 Matter. *Biological Reviews of the Cambridge Philosophical Society,* **63**, 433-462.
778 Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a
779 question of microbial competition? *Soil Biology & Biochemistry,* **35**, 837-843.
780 Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen
781 cycle: Recent trends, questions, and potential solutions. *Science,* **320**, 889-892.
782 Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in
783 density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic*
784 *Geochemistry,* **31**, 669-678.
785 Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of
786 benzenecarboxylic acids as specific markers. *Organic Geochemistry,* **29**, 811-819.
787 Goldberg ED (1985) *Black carbon in the environment: properties and distribution,* New
788 York, John Wiley and Sons.
789 Guo LB, Bek E, Gifford RM (2006) Woody debris in a 16-year old Pinus radiata
790 plantation in Australia: Mass, carbon and nitrogen stocks, and turnover. *Forest*
791 *Ecology and Management,* **228**, 145-151.
792 Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black
793 carbon and glucose mineralisation. *Organic Geochemistry,* **35**, 823-830.
794 Hammes K, Smernik RJ, Skjemstad JO, Herzog A, Vogt UF, Schmidt MWI (2006)
795 Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva)
796 and chestnut wood (Castanea sativa) as reference materials for black carbon
797 quantification. *Organic Geochemistry,* **37**, 1629-1633.
798 Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon
799 turnover observed in a Russian steppe soil. *Biogeosciences,* **5**, 1339-1350.
800 Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural
801 changes during the initial phase of microbial degradation of pyrogenic plant
802 residues in soil. *Organic Geochemistry,* **40**, 332-342.
803 Hilscher A, Knicker H (2011) Degradation of grass-derived pyrogenic organic material,
804 transport of the residues within a soil column and distribution in soil organic
805 matter fractions during a 28 month microcosm experiment. *Organic*
806 *Geochemistry,* **42**, 42-54.
807 Hockaday WC, Grannas AM, Kim S, Hatcher PG (2006) Direct molecular evidence for
808 the degradation and mobility of black carbon in soils from ultrahigh-resolution
809 mass spectral analysis of dissolved organic matter from a fire-impacted forest soil.
810 *Organic Geochemistry,* **37**, 501-510.
811 Hockaday WC, Grannas AM, Kim S, Hatcher PG (2007) The transformation and
812 mobility of charcoal in a fire-impacted watershed. *Geochimica Et Cosmochimica*
813 *Acta,* **71**, 3432-3445.
814 Jackson ML (1958) *Soil Chemical Analysis*, Prentice-Hall, Englewood Cliffs, New
815 Jersey, USA.

816 Janssens IA, Dieleman W, Luyssaert S *et al.* (2010) Reduction of forest soil respiration in
817 response to nitrogen deposition. *Nature Geoscience,* **3**, 315-322.
818 Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, Deluca TH (2011)
819 Short-term biochar-induced increase in soil CO2 release is both biotically and
820 abiotically mediated. *Soil Biology & Biochemistry,* **43**, 1723-1731.
821 Kammer A, Hagedorn F (2011) Mineralisation, leaching and stabilisation of C-13-
822 labelled leaf and twig litter in a beech forest soil. *Biogeosciences,* **8**, 2195-2208.
823 Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic Molecular Structure of
824 Plant Biomass-Derived Black Carbon (Biochar). *Environmental Science &*
825 *Technology,* **44**, 1247-1253.
826 Kloeti P, Keller HM, Guecheva M (1989) Effects of forest canopy on throughfall
827 precipitation chemistry. In: *Proceedings of a Symposium held during the Third*
828 *Scientific Assembly of the International Association of Hydrological Sciences.* pp
829 Page, Baltimore, Maryland, Atmospheric Deposition.
830 Knicker H (2011) Pyrogenic Organic Matter in Soil: Its Origin and Occurrence, its
831 Chemistry and Survival in Soil Environments. *Quaternary International,* **243**,
832 251-263.
833 Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: A
834 meta-analysis. *Ecology,* **86**, 3252-3257.
835 Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of Charcoal Quantity on Microbial
836 Biomass and Activity in Temperate Soils. *Soil Science Society of America*
837 *Journal,* **73**, 1173-1181.
838 Kuzyakov Y, Subbotina I, Chen HQ, Bogomolova I, Xu XL (2009) Black carbon
839 decomposition and incorporation into soil microbial biomass estimated by C-14
840 labeling. *Soil Biology & Biochemistry,* **41**, 210-219.
841 Kwon S, Pignatello JJ (2005) Effect of natural organic substances on the surface and
842 adsorptive properties of environmental black carbon (char): Pseudo pore blockage
843 by model lipid components and its implications for N-2-probed surface properties
844 of natural sorbents. *Environmental Science & Technology,* **39**, 7932-7939.
845 Laird DA, Chappell MA, Martens DA, Wershaw RL, Thompson M (2008)
846 Distinguishing black carbon from biogenic humic substances in soil clay
847 fractions. *Geoderma,* **143**, 115-122.
848 Lehmann J, Gaunt J, Rondon M (2006) Bio-Char sequestration in terrestrial ecosystems-
849 A review. *Mitigation and Adaptation Strategies for Global Change,* **11**, 403-427.
850 Lehmann J, Skjemstad J, Sohi S *et al.* (2008) Australian climate-carbon cycle feedback
851 reduced by soil black carbon. *Nature Geoscience,* **1**, 832-835.
852 Leifeld J, Fenner S, Muller M (2007) Mobility of black carbon in drained peatland soils.
853 *Biogeosciences,* **4**, 425-432.
854 Liang B, Lehmann J, Solomon D *et al.* (2008) Stability of biomass-derived black carbon
855 in soils. *Geochimica Et Cosmochimica Acta,* **72**, 6069-6078.
856 Liang BQ, Lehmann J, Sohi SP *et al.* (2010) Black carbon affects the cycling of non-
857 black carbon in soil. *Organic Geochemistry,* **41**, 206-213.
858 Mack MC, Schuur EaG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem
859 carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature,*
860 **431**, 440-443.

861 Major J, Lehmann J, Rondon M, Goodale C (2009a) Fate of soil-applied black carbon:
862 downward migration, leaching and soil respiration. *Global Change Biology,* **16**,
863 1366-1379.
864 Major J, Steiner C, Downie A, Lehamann J (2009b) Biochar effects on nutrient leaching.
865 In: *Biochar for Environmental Management. Science and Technology.* (eds J L,
866 Joseph S) pp Page. London, Earthscan.
867 Mccoll JG, Powers RF (1998) Decomposition of small diameter woody debris of red fir
868 determined by nuclear magnetic resonance. *Communications in Soil Science and*
869 *Plant Analysis,* **29**, 2691-2704.
870 Micks P, Downs MR, Magill AH, Nadelhoffer KJ, Aber JD (2004) Decomposing litter as
871 a sink for N-15-enriched additions to an oak forest and a red pine plantation.
872 *Forest Ecology and Management,* **196**, 71-87.
873 Moritz MA, Parisien M-A, Batllori E, Krawchuk MA, Dorn JV, Ganz DJ, Hayhole K
874 (2012) Climate change and disruptions to global fire activity. *Ecosphere,* **3**, 1-22.
875 Murage EW, Voroney PR (2007) Modification of the original chloroform fumigation
876 extraction technique to allow measurement of delta C-13 of soil microbial
877 biomass carbon. *Soil Biology & Biochemistry,* **39**, 1724-1729.
878 Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-
879 term black carbon dynamics in cultivated soil. *Biogeochemistry,* **89**, 295-308.
880 Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New*
881 *Phytologist,* **139**, 189-200.
882 Pietikäinen J, Kiikkila O, Fritze H (2000) Charcoal as a habitat for microbes and its effect
883 on the microbial community of the underlying humus. *Oikos,* **89**, 231-242.
884 Raveendran K, Ganesh A (1998) Adsorption characteristics and pore-development of
885 biomass-pyrolysis char. *Fuel,* **77**, 769-781.
886 Rovira P, Duguy B, Vallejo VR (2009) Black carbon in wildfire-affected shrubland
887 Mediterranean soils. *Journal of Plant Nutrition and Soil Science,* **172**, 43-52.
888 Ruehr NK, Buchmann N (2010) Soil respiration fluxes in a temperate mixed forest:
889 seasonality and temperature sensitivities differ among microbial and root-
890 rhizosphere respiration. *Tree Physiology,* **30**, 165-176.
891 Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006)
892 Black carbon contribution to soil organic matter composition in tropical sloping
893 land under slash and burn agriculture. *Geoderma,* **130**, 35-46.
894 Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in
895 temperate forest soils. *Soil Biology & Biochemistry,* **51**, 115-124.
896 Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: Analysis,
897 distribution, implications, and current challenges. *Global Biogeochemical Cycles,*
898 **14**, 777-793.
899 Schmidt MWI, Rumpel C, Kogel-Knabner I (1999a) Evaluation of an ultrasonic
900 dispersion procedure to isolate primary organomineral complexes from soils.
901 *European Journal of Soil Science,* **50**, 87-94.
902 Schmidt MWI, Skjemstad JO, Gehrt E, Kogel-Knabner I (1999b) Charred organic carbon
903 in German chernozemic soils. *European Journal of Soil Science,* **50**, 351-365.
904 Schmidt MWI, Torn MS, Abiven S *et al.* (2011) Persistence of soil organic matter as an
905 ecosystem property. *Nature,* **478**, 49-56.

906 Schneider MPW, Hilf M, Vogt UF, Schmidt MWI (2010) The benzene polycarboxylic
907 acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees
908 C. *Organic Geochemistry,* **41**, 1082-1088.
909 Schneider MPW, Lehmann J, Schmidt MWI (2011) Charcoal quality does not change
910 over a century in a tropical agro-ecosystem. *Soil Biology & Biochemistry,* **43**,
911 1992-1994.
912 Shen SM, Pruden G, Jenkinson DS (1984) Mineralization and Immobilization of
913 Nitrogen in Fumigated Soil and the Measurement of Microbial Biomass Nitrogen.
914 *Soil Biology & Biochemistry,* **16**, 437-444.
915 Shinogi Y, Yoshida H, Koizumi T, Yamaok M, Saito T (2003) Basic characteristics of
916 low-temperature carbon products from waste sludge. *Advances in Environmental*
917 *Research,* **7**, 661-665.
918 Singh N, Abiven S, Torn MS, Schmidt MWI (2012) Fire-derived organic carbon in soil
919 turns over on a centennial scale. *Biogeosciences,* **9**, 2847-2857.
920 Skjemstad JO, Reicosky DC, Wilts AR, Mcgowan JA (2002) Charcoal carbon in US
921 agricultural soils. *Soil Science Society of America Journal,* **66**, 1249-1255.
922 Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999) Soil organic carbon dynamics
923 under long-term sugarcane monoculture. *Australian Journal of Soil Research,* **37**,
924 151-164.
925 Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil
926 carbon balance and soil microbial activity. *Soil Biology & Biochemistry,* **41**,
927 1301-1310.
928 Steiner C, Das KC, Garcia M, Forster B, Zech W (2008) Charcoal and smoke extract
929 stimulate the soil microbial community in a highly weathered xanthic Ferralsol.
930 *Pedobiologia,* **51**, 359-366.
931 Stewart C, Zheng JY, Cotrufo MF (2011) Impact of biochar addition rate and soil type on
932 greenhouse gas emissions: The importance of long-term data. *Abstracts of Papers*
933 *of the American Chemical Society,* **242**.
934 Sudhakar Y, Dikshit AK (1999) Kinetics of endosulfan sorption on to wood charcoal.
935 *Journal of Environmental Science and Health Part B-Pesticides Food*
936 *Contaminants and Agricultural Wastes,* **34**, 587-615.
937 Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground
938 litter, belowground litter, and rhizosphere respiration to total soil CO2 efflux in an
939 old growth coniferous forest. *Biogeochemistry,* **73**, 231-256.
940 Topoliantz S, Ponge JF (2003) Burrowing activity of the geophagous earthworm
941 Pontoscolex corethrurus (Oligochaeta : Glossoscolecidae) in the presence of
942 charcoal. *Applied Soil Ecology,* **23**, 267-271.
943 Turunen J, Roulet NT, Moore TR, Richard PJH (2004) Nitrogen deposition and increased
944 carbon accumulation in ombrotrophic peatlands in Eastern Canada. *Global*
945 *Biogeochemical Cycles,* **18**.
946 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil
947 microbial biomass-C. *Soil Biology & Biochemistry,* **19**, 703-707.
948 Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011)
949 Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter
950 depletion in a Chernozem. *Soil Biology & Biochemistry,* **43**, 1985-1988.

951 Von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner
952 B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms
953 and their relevance under different soil conditions - a review. *European Journal of*
954 *Soil Science,* **57**, 426-445.
955 Wal A, Boer W, Smant W, Veen J (2007) Initial decay of woody fragments in soil is
956 influenced by size, vertical position, nitrogen availability and soil origin. *Plant*
957 *and Soil,* **301**, 189-201.
958 Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition
959 modifies soil carbon storage through changes in microbial enzymatic activity.
960 *Ecological Applications,* **14**, 1172-1177.
961 Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier
962 spring increase western US forest wildfire activity. *Science,* **313**, 940-943.
963 Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of
964 Soil Microbial Biomass C by Fumigation Extraction - an Automated Procedure.
965 *Soil Biology & Biochemistry,* **22**, 1167-1169.
966 Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved
967 organic matter in a temperate coniferous forest on Andic soils: Effects of litter
968 quality. *Ecosystems,* **8**, 286-300.
969 Yarnes C, Santos F, Singh N, Abiven S, Schmidt MWI, Bird JA (2011) Stable isotopic
970 analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-
971 ratio mass spectrometry of benzene polycarboxylic acids. *Rapid Communications*
972 *in Mass Spectrometry,* **25**, 3723-3731.
973 Zalamea M, Gonzalez G, Ping CL, Michaelson G (2007) Soil organic matter dynamics
974 under decaying wood in a subtropical wet forest: effect of tree species and decay
975 stage. *Plant and Soil,* **296**, 173-185.
976 Zavalloni C, Alberti G, Biasiol S, Delle Vedove G, Fornasier F, Liu J, Peressotti A
977 (2011) Microbial mineralization of biochar and wheat straw mixture in soil: A
978 short-term study. *Applied Soil Ecology,* **50**, 45-51.
979 Zell J, Kandler G, Hanewinkel M (2009) Predicting constant decay rates of coarse woody
980 debris-A meta-analysis approach with a mixed model. *Ecological Modelling,* **220**,
981 904-912.
982 Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black
983 carbon (biochar). *Environmental Science & Technology,* **44**, 1295-1301.
984 Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization
985 priming effects among a variety of biochar-amended soils. *Soil Biology &*
986 *Biochemistry,* **43**, 1169-1179.
987
988
989
990
991
992
993
994
995
996

997

998 **Table 1**: Physical and chemical characteristics of the soil in the 0 to 15 cm depth. Values correspond to the mean (n=3) and values
999 between brackets correspond to standard errors.
1000

Texture			**Bulk density**			**pH**	**CEC**	**Elemental analysis**										
%			g cm^{-3}				mmol kg^{-1}	g kg^{-1} soil										
Sand	Silt	Clay	0-5 cm	5-10 cm	10-15 cm			C	H	N	Na	Mg	Al	Si	P	K	Ca	Mg	Fe
45.5 (2.0)	24.2 (2.5)	31.5 (1.4)	1.20 (0.1)	1.21 (0.2)	1.60 (0.2)	5.9 (0.3)	74.3 (8.6)	33.7 (2.8)	8.9 (0.4)	2.4 (0.1)	7.8	11.2	71.6	317	0.3	19.5	4.6	1.4	32.8

1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021

Table 2: Recovery of applied $^{13}C/^{15}N$-labeled wood or PyOM and excess ^{13}C and ^{15}N in the soil at depths of 0-5 cm, 5-10 cm and 10-15 cm and bulk soil, 10 months after application. The initial C:N ratio of added PyOM was 110 and wood was 115. Different letters in the same column (within the same depth) are significantly different ($p \leq 0.05$).

Substrate	**Soil depth**			**Bulk soil**	**Excess ^{13}C, mg kg^{-1} soil**		
	0-5 cm	5-10 cm	10-15 cm		0-5 cm	5-10 cm	10-15 cm
^{13}C-substrate recovered (% of the applied after 10 months)							
Wood, N0	40.0 (13.2)[a]	6.5 (3.9)[a]	1.6 (0.9)[a]	48.2 (4.6)[a]	14.7 (5.3)	2.0 (1.1)	0.5 (0.3)
Wood, N+	44.5 (5.1)[a]	2.1 (0.3)[a]	1.8 (0.9)[a]	48.4 (8.8)[a]	14.5 (3.0)	0.7 (0.1)	0.5 (0.2)
PyOM, N0	95.1 (1.7)[b]	3.1 (0.9)[a]	1.1 (0.7)[a]	99.4 (0.3)[b]	64.0 (8.5)	1.5 (0.3)	0.5 (0.2)
PyOM, N+	96.5 (1.4)[b]	2.3 (0.7)[a]	1.1 (0.9)[a]	99.9 (0.4)[b]	71.2 (5.8)	1.5 (0.5)	0.5 (0.4)
^{15}N-substrate recovered (% of the applied after 10 months)					**Excess ^{15}N, mg kg^{-1} soil**		
	0-5 cm	5-10 cm	10-15 cm	Bulk	0-5 cm	5-10 cm	10-15 cm
Wood, N0	78.2 (3.8)[a]	7.1 (2.8)[a]	3.1 (0.8)[a]	88.5 (6.4)[a]	0.9 (0.05)	0.1 (0.02)	0.03 (0.01)
Wood, N+	35.1 (7.5)[b]	7.8 (0.8)[a]	5.1 (2.0)[a]	48.0 (6.0)[b]	0.4 (0.12)	0.1 (0.00)	0.05 (0.03)
PyOM, N0	81.5 (2.6)[a]	6.1 (2.0)[a]	2.0 (1.0)[a]	89.2 (0.4)[a]	2.0 (0.32)	0.1 (0.04)	0.03 (0.02)
PyOM, N+	89.2 (2.3)[a]	5.8 (0.9)[a]	2.7 (0.2)[a]	97.7 (3.0)[a]	2.4 (0.21)	0.6 (0.46)	0.05 (0.01)

Table 3: Total C and N recovery of bulk soil and added substrate after density fractionation. Values correspond to the mean (n=3) and values between brackets correspond to standard errors.

Treatments	Total C recovery, %		Total N recovery, %	
	Bulk soil	Added substrate	Bulk Soil	Added substrate
Wood, N0	88.6 (18)	78.8 (1)	93.0 (17)	45.2 (14)
Wood, N+	91.4 (4)	67.4 (17)	85.0 (5)	94.7 (14)
PyOM, N0	88.4 (1)	102.5 (10)	83.0 (2)	103.9 (10)
PyOM, N+	90.4 (3)	106.3 (2)	85.2 (2)	97.8 (9)
Control, N0	86.1 (7)		85.5 (2)	
Control, N+	88.2 (3)		77.6 (5)	

1060 **Table 4:** Total yield (% of soil), total C and N in SOM fractions (g kg^{-1} fraction) and bulk soil (g kg^{-1} soil) from 0-5 cm depth. Values
1061 correspond to the mean (n=3) and values between brackets correspond to standard errors.
1062

Treatments	**Density Fractions (g cm^{-3})**									**Bulk soil**	
	fLF			oLF			DF				
	Yield	C	N	Yield	C	N	Yield	C	N	C	N
	% of soil	g kg^{-1} fraction		% of soil	g kg^{-1} fraction		% of soil	g kg^{-1} fraction		g kg^{-1} soil	
Wood, N0	1 (0.1)	299 (21)	9 (0.8)	1 (0.3)	393 (17)	12 (0.5)	98 (0.3)	20 (2)	2 (0.1)	30 (5)	2 (0.3)
Wood, N+	3 (0.2)	296 (14)	10 (0.5)	2 (0.3)	404 (8)	14(0.5)	99(0.5)	32 (2)	2 (0.2)	48 (2)	3 (0.2)
PyOM, N0	2 (0.5)	394 (19)	8 (1.6)	2 (0.3)	421 (27)	13 (2.2)	96(0.9)	21(2)	2 (0.2)	41(6)	2 (0.3)
PyOM, N+	3 (0.2)	387 (32)	8 (0.1)	3 (0.3)	428 (5)	14 (0.5)	95 (0.5)	23 (3)	2 (0.2)	45 (3)	3 (0.2)
Control, N0	1 (0.2)	288 (28)	11 (0.3)	2 (0.1)	393 (9)	15(0.3)	97 (0.2)	24 (2)	2 (0.2)	36 (4)	2 (0.2)
Control, N+	1 (0.2)	297 (13)	11 (0.3)	2 (0.3)	405 (1)	16 (0.5)	97 (0.5)	24 (3)	2 (0.1)	36 (2)	3 (0.1)

1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079

1080 **Table 5**: Microbial biomass 10 months after $^{13}C/^{15}N$-labeled wood or PyOM addition to soil mesocosms across all N treatments.
1081 Values correspond to the mean (n=6) and values between brackets correspond to standard errors.

Treatment	Soil microbial biomass C = $(C_{fum}-C_{nfum})/0.45$, μg g^{-1} dry soil			Soil microbial biomass N = $(N_{fum}-N_{nfum})/0.68$, μg g^{-1} dry soil			Excess ^{13}C in microbial biomass μg g^{-1} dry soil			C:N ratio of soil microbial biomass		
	0-5 cm	5-10 cm	10-15 cm	0-5 cm	5-10 cm	10-15 cm	0-5 cm	5-10 cm	10-15 cm	0-5 cm	5-10 cm	10-15 cm
Wood	699 (86)	581 (86)	427 (86)	70 (11)	87 (6)	51 (8)	0.22 (0.05)	0.23 (0.15)	0.02 (0.01)	9.8 (2.3)	6.9 (1.9)	8.8 (2.1)
PyOM	671 (77)	454 (77)	360 (86)	79 (27)	42 (8)	32 (7)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	8.4 (4.2)	10.9 (3.3)	11.4 (3.7)
Control	661 (77)	496 (77)	417 (94)	78 (10)	63 (7)	12 (4)	-	-	-	8.5 (1.5)	7.9 (1.9)	31.8 (13.2)

1082
1083 ** Indicates significant difference ($p<0.05$) in SMBC with depth for each treatment*
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093

1094 **Figure legends**

1095

1096 **Figure 1**: Distribution of ^{13}C excess derived from wood or PyOM among SOM fractions
1097 (fLF: free light fraction; oLF: occluded light fraction and DF: dense fraction) 10 months
1098 after organic substrates application to the soil. SOM fractions shown are from 0-5 cm soil
1099 depth. The values correspond to the mean (n=3) and the bars to the standard error.

1100

1101 **Figure 2**: Relative changes of native SOC among SOM fractions (fLF, oLF and DF) and
1102 bulk soils 10 months after addition of $^{13}C/^{15}N$-labeled wood or PyOM, expressed as
1103 differences from control plots. The error bars represent standard errors, n=3.

1104

1105 **Figure 3**: Relative contributions of individual BPCA marker molecules (left, y-axis as
1106 bars) and total BPCA-C contents (right, y-axis as circles) in fresh PyOM, PyOM mixed
1107 with soil before (t =0) and 10 months after the experiment started under ambient N (N0)
1108 and increased N (N+) treated soils. Different letters indicate significance difference
1109 ($p<0.05$). The values correspond to the mean (n=3) and the bars to the standard error;
1110 B3CA = benzene tri-carboxylic acid, B4CA = benzene tetra-carboxylic acid, B5CA =
1111 benzene penta-carboxylic acid, B6CA = benzene hexa-carboxylic acid.

1112

1113

1114

1115

1116
1117

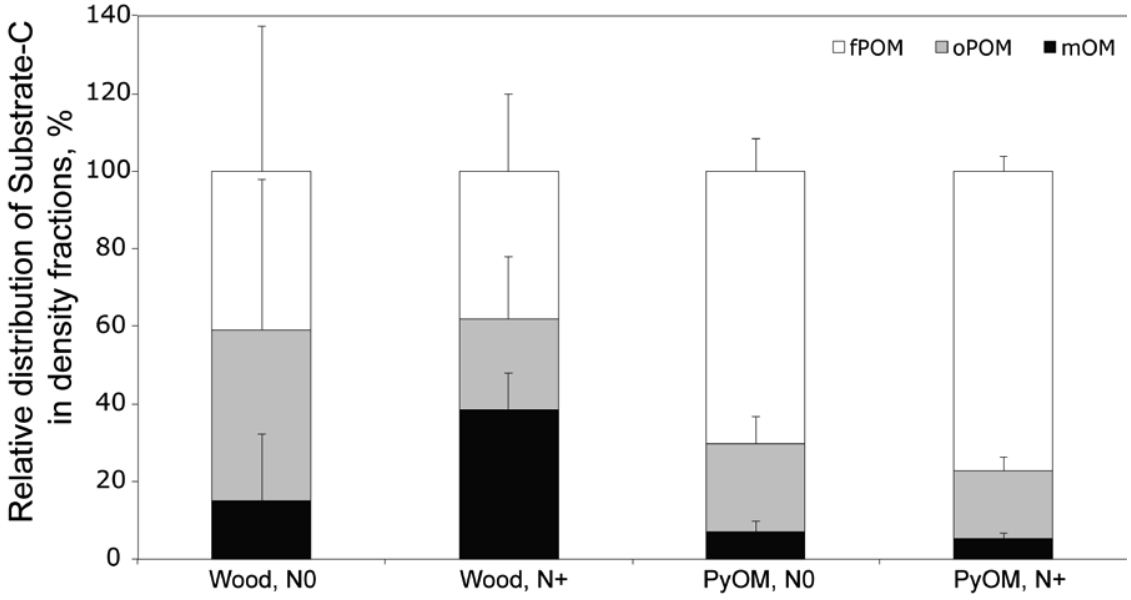

Relative distribution of Substrate-C in density fractions, %
140
120
100
80
60
40
20
0
fPOM
oPOM
mOM
Wood, N0
Wood, N+
PyOM, N0
PyOM, N+

Figure 1

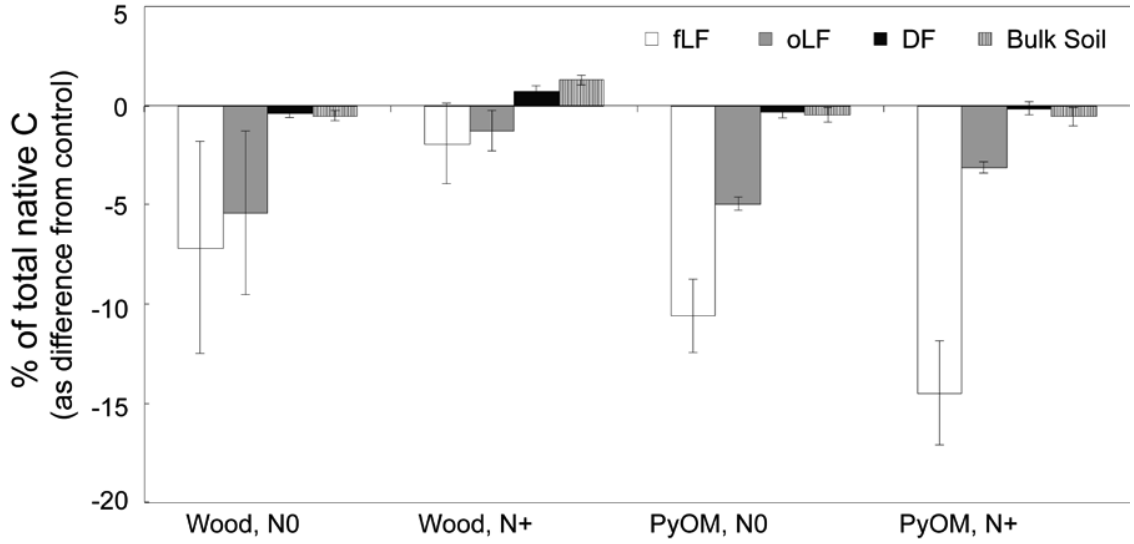

5
0
-5
-10
-15
-20
% of total native C
(as difference from control)
fLF
oLF
DF
Bulk Soil
Wood, N0
Wood, N+
PyOM, N0
PyOM, N+

Figure 2

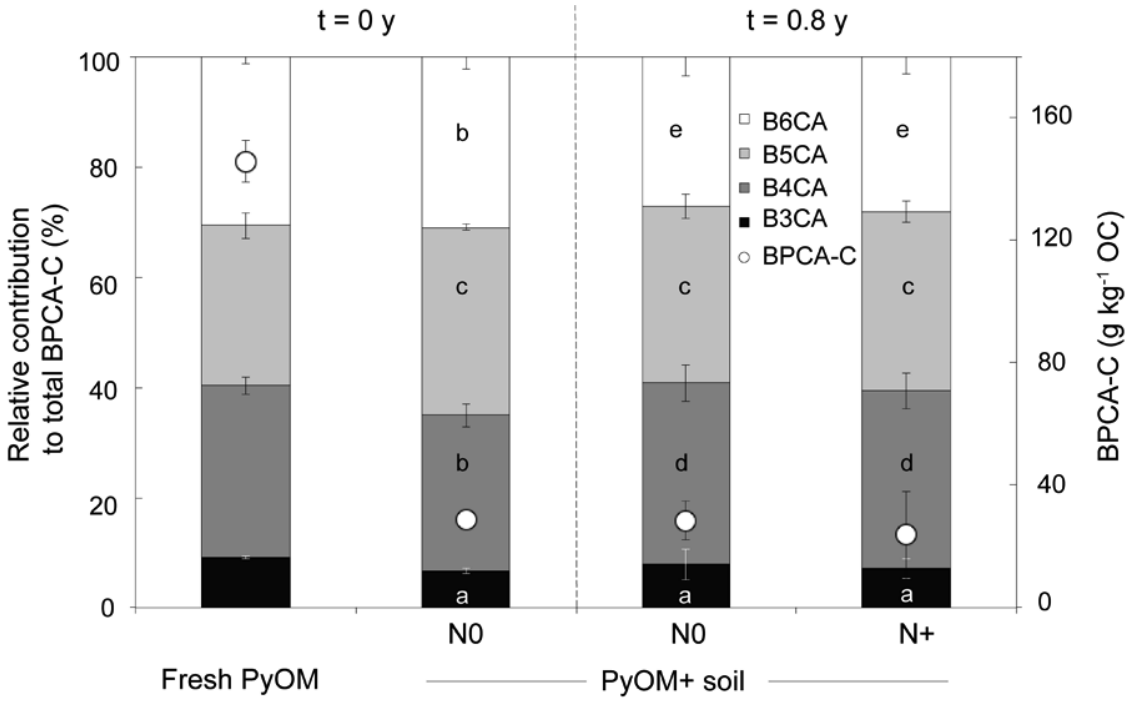

t = 0 y
t = 0.8 y
Relative contribution to total BPCA-C (%)
BPCA-C (g kg-1 OC)
B6CA
B5CA
B4CA
B3CA
BPCA-C
N0
N0
N+
Fresh PyOM
PyOM+ soil

Figure 3

Chapter C

Appendix

Curriculum Vitae

Maestrini Bernardo
Winterthurerstrasse, 190
CH-8057, Zurich, Switzerland

Phone: 0041 (0)44 6355227
Mobile: 0041 (0)76 7695965
Email: bernardo.maestrini@geo.uzh.ch
Homepage: http://tiny.uzh.ch/3w

*27.03.1984
Italian
Swiss driving licence: B

Education

09/2009-to date	**University of Zurich**, PhD student PhD topic: Pyrogenic organic matter decomposition in soil Teaching lectures and field classes in soil science courses Skills: scientific presentations, scientific writing, project management Expected dissertation: December 2013
2006-2009	**University of Padua**, M. Sc. Forestry Master thesis topic: Policies against desertification in Syria in collaboration with GCSAR institute,Damascus Specialization: Forest policies and economy
2003-2006	**University of Florence**, B. Sc. Forestry

Publications

12/2013	Maestrini B., Herrmann A. M., Nannipieri P., Schmidt M.W.I., Abiven S., Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil, Soil Biology and Biochemistry,69:291-301 (2014)
12/2013	Maestrini B., Abiven S., Singh N., Bird J. A., Torn M. S., Schmidt M. W. I., Carbon losses from pyrolysed and original wood in a forest soil under natural and increased N deposition, Discussion Article in Biogeosciences.
10/2013	Singh N., Abiven S.,Maestrini B., Bird J. A., Torn M. S. and Schmidt M. W. I., Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment, Global Change Biology, published online (doi: 10.1111/gcb.12459)
1/2014	*Maestrini B.*, Nannipieri P. Abiven S., Pyrogenic induced priming effect on soil organic matter, accepted for publication in Global Change Biology - Bioenergy.

Conferences

10/2013	Biochar, Compost and Digestate (BCD) conference,Bari,oral presentation
02/2013	Swiss Soil Science Society General Assembly, Zurich, oral presentation
07/2013	Eurosoil,Bari, oral presentation
04/2012	European Geophysical Union,Vienna, poster presentation
04/2011	European Geophysical Union,Vienna, oral presentation
11/2010	Swiss Geoscience Meeting, Fribourg,oral presentation
05/2010	European Geophysical Union, Vienna, oral presentation

Grants and fundings

2013	Swiss National Science Foundation, Individual post-doc grant at the Michigan State University, Prof. J. Miesel (18 months)
2013	Italian Soil Science Society, grant for BCD conference 2013
2011	University of Zurich, InnoPool funding scheme: Lieberherr S., Singh N., Maestrini B., Abiven S. Feasibility study on the introduction of Biochar in Indian villages

Professional experiences

06-08/2009	**Studio Gardin**, Florence, collaboration Soil mapping (through remote sensing and field validation)
07-08/2005	**Research Institute for Forest Management** , Arezzo, Internship Measurement of wood production in intensively managed forests

Courses

05/2011	**Centro Geotecnologie**, Siena,WebGis Open-Source(WGOS)
04/2011	**University of Zurich** Using R for statistical data analysis and graphics
01/2011	**Etifor**, Padova, The forest management auditor of tomorrow
01/2010	**Paul Scherrer Institute**, Villingen, Introduction to Stable Isotopes in Plant Ecological Physiology
04/2009	**University of Lublin**, Role of Agriculture in Territorial Identity and Competitiveness of rural areas
06/2008	**University of Lubljana** , Plant biotechnologies and applications,
07/2008	**University of Lubljiana**, Management of water quality.

Acknowledgments

A number of people deserve an acknowledgment for their contribution direct or indirect to this thesis. First of all I want to thank my supervisor Dr. Samuel Abiven, for the enthusiasm he always transmitted me, his support and openness to new ideas. I think the passion he shows for science helped me to feel deeply involved in the project. A special mention goes of course also to his friendship and *savoir faire* beyond science, that has been invaluable since the very beginning. I want to thank Prof. Michael Schmidt, for his mentoring activity, particularly for always keeping the direction and the situation of my PhD under control. I learned a lot from his experience on the functioning of the scientific world.
Thanks to Prof. Paolo Nannipieri, for accepting to be part of my PhD committee. His scientific inputs were always extremely useful, practical and prompt. I particularly appreciate the carefulness of his revisions and the broadness of his scientific considerations. Thanks also to the co-authors of my manuscripts: Prof. Jeffrey Bird, for reading my manuscript a number of times. His carefulness for details pushed me to dig always a bit deeper. Eventually that helped me a lot, especially for statistics. So I really appreciate all his efforts in our work. Thanks also to Prof. Margaret Torn for the numerous, constructive and original comments on the manuscripts and the fieldwork. Thanks to Dr. Anke Herrmann for hosting me in her laboratory in Uppsala and for teaching me the isotope dilution technique, thanks for the time she dedicated to me and for her willingness to help with calculations, laboratory work and data interpretation.
Thanks to the people of Paul Scherrer Institute, Dr. Matthias Saurer, Dr. Catharina Lötscher, and Prof. Rolf Siegwolf. Their collaboration has always been prompt and extremely competent.
Thanks to the laboratory technicians of the 2B group, Michael Hilf, who always surprises me for his capacity to conjugate order and creativity, Sandra Roethlisberger, Claudia Schreiner, and Bruno Kägi. A special thank to Ivan Woodhatch for his smart solutions and jokes.
Thanks to Elizabeth Nietlispach, Helen Grter, Lukas Japp and Ljubica Lindov for all the administrative work, and for being always been helpful and patient.
Thanks to the students who helped me during field and laboratory work, they have always been very careful in their work and full of ideas on how to improve the methods: Fabian Zust, Marisa Brauchli, Charlotte Huber, Verena Lanny, Stefanie Müller, Julia Siegrist, Annika Tella, Barbara Bachman, Lacey Pile, Cecile Wanner, Rmi Albinet. Thanks to Nimisha, with whom I closely collaborated for the Laegeren project, she has taught me really a lot especially about laboratory. Thanks also for all the chat and support I received from her. I

particularly appreciate her sense of humour, especially in the field. Thanks to Mirjam for her help, especially with isotopes. I enjoyed a lot all the occasions outside university, from skiing to turnip festivals.
Thanks to all the colleagues and visitors of the 2B group and of the geography department for the fruitful scientific discussion, and the nice time spent together outside university: particularly Daniel, Marco, Beatriz, Kavita, Barbara, Anett, Dolores, Max, Guido, Lucie, Lucia, Petra, Christian, Filippo, Marta, Marc, Maria, Michael, Andrew, Nadezda, Fernanda, Suzanne, Alice.
A special thank goes to Benjamin, with whom I have the luck to share the office and a good friendship and also to Ilaria of course. Thanks also to Dr. Paolo Cherubini for all the discussions on science and beyond and also for the little work we have done together. Thanks to Dr. Giacomo Certini, for his friendship and for having initiated all this, thanks also for the scientific and non-scientific discussions. Thanks also to Gianni for his help in many situations, I always appreciate his sense of humour, it makes everything look lighter. Thanks to the friends of the Fabbrica di Zurigo, specially Mattia and Steve, two end members of my personal idealism-pragmatism mixing model, Ivan, Marco, Roberto, Gaia, Michela, Valeria, Manuela. Thanks to Mario Mantovani who long back pushed me toward hard sciences, eventually I do not regret it.
A special thank goes to my family, my mother, my father and my sister who never restricted me while rather always encouraged me. Thanks finally to Daniela for supporting and *sopporting* me :-) .